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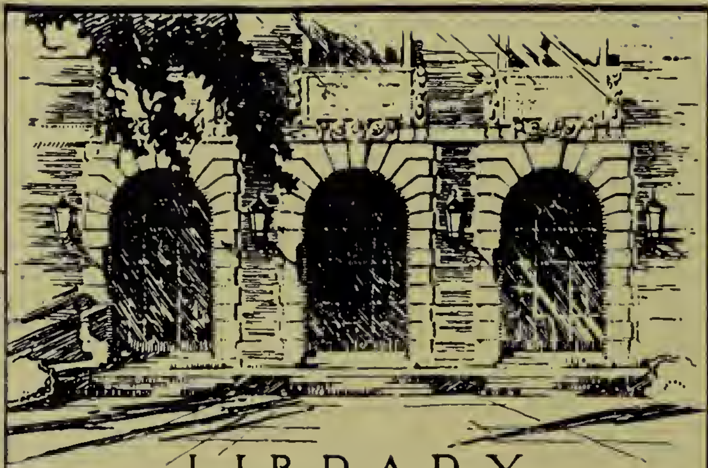

XI. Internationaler Kongreß für Entomologie

Wien, 17. bis 25. August 1960

VERHANDLUNGEN

Band III
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SYMPOSIUM I

INSEKTEN-AKUSTIK

PROGRESS AND PROBLEMS IN INSECT ACOUSTICS

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It is a very great honour and pleasure for me this afternoon to open this Symposium, the first international meeting ever held covering the acoustics of all insects. Because of the nature of the occasion my colleagues and I have thought it proper to try and cover a very broad field in the programme, referring not only to work in progress but to work completed. Insect acoustics has been the subject of comment and observation for a very long time from Aristotle onwards but it is only in fairly recent years that detailed experimental work has allowed a framework of theory to be erected, covering, for some species, production and reception of sounds and behaviour associated with it. It is, I think, particularly appropriate that this first international meeting should be held in Vienna, because here in the Sophiengymnasium worked a man whose experimental approach laid the basis for much future work—Professor D. J. Regen. Following Regen's experimental work came Professor Albrecht Faber in Germany whose detailed observations on the relationship of song and behaviour in grasshoppers laid the foundations for most of todays acoustic behaviour work. I am very glad that Professor Faber is here to-day participating in this Symposium.

Despite the progress that has already been made, there seems to be a tendency for entomologists investigating problems of insect behaviour to overlook the possible role of acoustic signals in that behaviour and part of the purpose of this symposium is to introduce this international audience to a selection of the wide variety of situations in which such signals may be involved. I have so far purposely avoided using the phrase "sound signals" since this has implicit in it the suggestion of signal frequencies audible to man; certainly insects produce and use sounds of these frequencies but also they employ signals in the infra- and ultra-sonic range, as later papers will show. Furthermore—and this is an important point—I include in the term "acoustic signal" stimuli transmitted by vibrations of the substrate. As we shall see, insects can transmit and receive such vibrations and this phenomenon certainly plays a part in several behaviour patterns. Information such as that I have just mentioned on the frequency range of insect sound signals has been obtained in a wide-ranging series of investigations which has included recording and analysis of the sounds themselves, study of the mechanisms by which they are produced and detected and to a lesser extent the immediate behaviour in which they are involved. Little attention has as yet been paid to the problem of linking the morphological and physiological components of the sound system with other systems of the insect or its general biology, and in this paper, partly as a background to the remaining papers and partly to direct attention to the need and opportunity for research which this aspects presents, I shall briefly consider four fields of work, themselves inter-related, which bear on this point. These fields are defensive and warn-

ing behaviour, sexual behaviour, sounds related to organisation in social and sul social groups and the rôle of acoustics in speciation.

It is a common-place observation that many insects produce noises on being touche or handled and many workers have characterised such noises as "alarm" or "danger calls. Amongst the Heteroptera and Coleoptera many species only stridulate whe touched and never appear to produce their noises spontaneously. It has been held th such behaviour may serve to protect an insect if it is seized by a predator such as a bir or spider, which may be so startled by the ensuing noise that it will let go its hold. A Pumphrey (1955) has pointed out, it is possible to regard such noises as in the sam category of extra-specific stimuli as "warning colours" with which, incidentally, the may work in conjunction. The necessary conditions for this reaction to be useful ar that the noise produced shall be of a frequency and intensity such as to stimulate th sound receptors of the predator, or preferably a range of predators. To stimulate a large a range of predator hearing organs as possible, it is preferable for the insec sound to be a "noise" in the correct sense of the word—that is a signal with a ver wide frequency spectrum and without recognizable pattern. Here is an example of suc a noise produced by the bug *Coranus apterus* (Haskell, 1957); the duration and intensit of the sound depends on these qualities of the stimulus. Now many beetles and bug produce noises only on tactile stimulation, and because of this these sounds have bee characterised as "defense noises". Here is a recording of such a noise from the beetl *Prionus coriarius*. But in several species which I have investigated the sound emitted ha none of the characters of a warning noise, but has all those necessary in a communicativ signal. Here, for example, is a recording of the sound of the beetle *Lilioceris lilii*; notic the organised pattern of pulses occurring at a fixed rate. The signal emitted is also th same regardless of the strength of the tactile stimulus. Now this signal is produce in a group of *Lilioceris* when one insect touches another; and when one considers that thi species is strictly confined to one host plant and for that reason is often found in quit dense groups, there is some ground for thinking the sounds may in this case be intra specific.

Consider now those subterranean coleopterous larvae which produce sounds; som of them—notably the Passalids—are so severely adapted for sound production that th idea that this fully functional apparatus is a useless mutation seems ridiculous. Bu what purpose does it serve? Clearly not a sexual one and therefore, it has been suggested it is defensive—possibly against moles and other burrowing predators. But the difficult here is that the sounds are, in some species at least, produced spontaneously, an un desirable feature in a warning sound. Now many of these coleopterous larvae are found in groups in the soil; and it may well be that pupation and consequent emergence o the adults close together may be advantageous, in, for example, promoting meeting of the sexes. These examples serve to show that in this aspect of insect acoustics, as in others, facile behavioural interpretation without full knowledge of the biologica background is to be avoided.

In the realm of sexual behaviour the same sort of over simplification has occurred; it has been said by many entomologists that the sole purpose of insect sounds is to bring together the sexes for mating, and various incorrect statements are made in support of this contention, the favourite one being, in the case of Orthopteroid insects, that only the males can sing. Not only is this untrue but behavioural investigation has shown that the song of the female often plays a vital rôle in sexual behaviour, and the same has been found to hold true in several species of Heteroptera. One of the earliest and apparently most complete acoustico-sexual patterns to be investigated was the attraction of the male of *Aedes aegypti* by the flight note of the female. Roth (1948) worked out this story and the fact that he could get male *Aëdes* to respond either to sounds made by

flying females or to a tuning fork giving out the same note seemed to demonstrate convincingly the value of the sound stimulus in bringing together the sexes. But the recent careful work of Wishart and Riordan (1959) while supporting Roth's general finding has raised some puzzling points. Males can distinguish the attractive sound of the female flight note in the presence of very high background noise—up to as much as 100 times as loud as the signal—but if two sounds which are attractive alone are presented together the response virtually disappears. This implies that attraction of a male to a female can only occur in relative isolation and not in crowds or swarms. Again, some basic biological research is required.

While we can now with some certainty describe the general course of events in the acoustic initiation and stimulation of sexual behaviour in many species of the Orthoptera, Heteroptera and Diptera we are as yet almost entirely ignorant of the modes of interaction between their reproductive and acoustic systems. But linked they must be; rates of maturation, development of eggs, oviposition—all these functions of the reproductive system must integrate with the operation of the sound system. What evidence we do have suggests that the linkage varies both according to the group and the sex. The males of Acrididae, for example, sing their "normal" song when immature, and resume it an hour or so after a complete mating, irrespective of the presence or absence of sperm in their gonads. But in Tettigoniids and Gryllids the males only sing when both testes and accessory glands are replete with material for producing a spermatophore, and immediately after copulation and the discharge of this spermatophore they are silent for a period of days until a new spermatophore is ready. The work of Huber (1952) has shown the rôle of the nervous system in this mechanism in the cricket *Gryllus campestris*; the formation of a spermatophore is signalled by unknown receptors in the genital segments to the corpora pedunculata of the brain, with the consequent release of song behaviour.

The relationships in females are even more complicated; Regen showed as early as 1913 that only adult but unmated females of *Gryllus campestris* would react to male "normal" song by moving toward the source of sound. The work of Renner (1952) and Haskell (1958a) has amplified this finding for several species of Acrididae. The female grasshopper is unresponsive to male song from the time of its final moult until it is sexually mature, or more precisely until the first eggs are ready to pass into the oviducts. It then responds to male "normal" song, which elicits the typical behaviour of orientation to and locomotion towards the source of sound. In this state females will allow males of their own species to copulate without the preamble of a "normal" song, provided the male sings the "courtship" song. But once copulation is complete, the female becomes unresponsive to male song. In *Euthystira brachyptera*, Renner found this state was terminated by oviposition, but Haskell found with other grasshoppers that the females rarely became responsive again provided they were able to hear male normal song, but that acoustic isolation secured a return to the responsive state. Both Renner and Haskell found that the responsive state of the female was terminated by approaching oviposition; the response disappeared about 24 hours before oviposition and returned 2—3 hours after it.

In the case of termination of the responsive state by copulation, such evidence as is available suggests that some chemical factor associated with the sperm is involved in the inhibition. This is so for Tettigoniids (Busnel et al., 1956) and Acridids (Haskell, 1960). The time element in much of this behaviour suggests hormonal action, and very recently Haskell (1960) was able to induce the responsive state in castrated female grasshoppers by injection of blood from normal females in the responsive state. Examination of the evidence from many groups of insects underlines this same close link between maturation and song, but the precise mode of integration of the acoustic system with reproductive behaviour awaits further physiological work.

It has been widely assumed that the rôle of acoustic signals in most Orthoptera is entirely and immediately subservient to sexual behaviour, but Haskell (1958b) has adduced evidence to show that gregariousness and colony cohesion in certain grasshoppers can be attributed at least partly to sound signals. In other groups, for example Cicadas, there is evidence to support the idea that sound signals lead primarily to the formation of dense aggregations of the insects and that reproductive contact is a secondary outcome of such grouping. Such findings naturally lead us on to consider the part played by sound signals in the truly social insects; here, until recently, the wealth of anecdotal and circumstantial evidence was unsupported by experimental work, but Lindauer and Kerr (1960) have now concluded that communication between certain species of stingless bee is at least partly acoustic. Returning scout bees of *Melipona scutellaris*, *Trigona postica* and *T. ruficrus* gave small amounts of syrup to nearby bees in the hive and then ran irregularly amongst them in a zig-zag pattern, stimulating them by touch, and at the same time emitting a characteristic buzzing sound. The sound was made at the source of food, on entering the hive and while in it. The sound in *Melipona quadrifasciata* was loud and characteristic enough to be detected 1½ metres away. Behaviour work showed that scouts could stimulate other bees without physical contact, provided the buzzing was heard. Buzzing stopped immediately food was withdrawn. The rhythm of the sound was different for all seven species investigated. Experiments with hives lined with foam rubber and galvanised iron respectively suggested that the stimulus was picked up by the sub-genual organs in the legs of the bees—that is, they were responding to the vibrations of the substrate associated with the production of the buzzing noise, rather than directly to the air-born sound itself. Finally, of great interest is the fact that when two attempts were made to rear a colony of *M. quadrifasciata* in a sound proofed hive lined with cellotex both experiments failed, the colonies gradually dying out. Lindauer and Kerr say "it may be that transmission of vibrations is an essential quality for a Meliponini hive".

It is of course well known that honey bees also make noises, both workers and queens. The source of sound and its significance has been bitterly disputed and I do not intend to enter into this controversy here. However, the problem is being studied by several workers and Mr. A. M. Wenner of the Department of Entomology, University of Michigan, has very kindly made some of his recent results available to me to show you. These are audio-spectrographs of recordings of the sounds. No. 1 is a worker "piping"; this behaviour appears transitorily in the hive, usually only in one bee at a time. No. 2 is from an individual worker which has been intensely disturbed. The pattern varies with the degree of disturbance. No. 3 is from a single bee engaged in ventilating the hive. No. 4 is from a bee carrying out the communication dance after a flight of about 500 metres. No. 5 is of a queen piping. No. 6 is an expanded train of pulses from the sound of a dancing bee to show the pulsed nature of the sounds. I present this information to you with the sole comment that it seems to me most unlikely that the nervous and muscular adaptations necessary to produce these well differentiated patterns would have evolved without some usefulness to the insect and the colony.

Finally, I must mention the possible rôle of sound signals in evolution and speciation. As far as direct survival value is concerned there are two main aspects to be considered; one is the possibility that insects can frighten off or escape from predators by the use of sounds and the second is that by use of sound signals meetings of the sexes are brought about and mating facilitated. A third aspect of the survival value of sound signals, which may be thought of as indirect, is its effects on the ecology of the species.

It should be said at once that there is very little, if any, direct evidence of the power of stridulation in frightening off predators; but the widespread evolution of pupal stridulation in the Lepidoptera for example, which can apparently have no possible

significance other than defense (Hinton, 1948), and the audio mimicry of wasps by certain flies (Gaul, 1952) suggests that under some conditions sound can have a defensive and hence survival value; the field is a fascinating one and open wide for research.

On the aspect of facilitation of courtship and mating a great deal of evidence is available and it is no longer possible to doubt that in several groups of the Orthoptera and probably the Hemiptera and Diptera as well meetings of the sexes and epigamic display leading to mating is initiated and controlled by acoustic stimuli. Further than this at present it seems unwise to go, for although it is widely agreed that reproductive isolation has definite survival value, evidence for this in respect of insect sounds must derive from cases where it can be clearly shown that the sole or principal isolating mechanism is song specificity and song discrimination. At present such evidence is available for one or two species, although the probability exists in many more. The work of Perdeck (1957) has put it beyond reasonable doubt that the grasshoppers *Chorthippus brunneus* and *C. biguttulus* are isolated solely by virtue of their different song patterns; other work on crickets, cicadas and certain bugs is suggestive but not so critical.

Lastly, song behaviour may effect distributional ecology and hence have survival value; several species of crickets show "territorial song behaviour" very like that of birds; cicadas and some bugs show aggregating behaviour in relation to sound; and finally in certain grasshopper "colonies" gregariousness may be maintained by acoustic methods. Such data can only be regarded as the first steps towards a fuller knowledge of the part played by acoustics in speciation and evolution and further evaluation of this rôle depends not only on further research into insect sounds but also on the fitting of this knowledge into an extended background of information on the general biology of the species concerned. Of course there will always be the need for specialist studies of the organs of production and reception of sounds, the physiology of these processes and the integration of the acoustic system into the general functional framework of the insect organism. But perhaps above all to advance the study of insect acoustics we need to make it clear that the production or reception of acoustic signals through the air, the ground or water is not an occasional phenomenon in the insect world, to be considered reluctantly or as inherently improbable, but as a very widespread occurrence, encompassing at least twelve insect orders and playing its part in a very wide range of behaviour patterns. It is clearly impossible in the present context to survey adequately this field; I have tried here to sketch in some of the broad outlines, to indicate the nature of some of the research required and lastly to give some sort of background against which the remainder of the papers in this Symposium can be considered.

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FUNDAMENTAL QUESTIONS OF COMPARATIVE RESEARCH IN BIOACOUSTICAL PHENOMENA AMONG INSECTS; ACOUSTICAL FORMS OF EXPRESSION, THEIR DEVELOPMENT AND THEIR SIGNIFICANCE

A. FABER

Manuskript und Abstract nicht eingelangt

A PRELIMINARY CLASSIFICATION OF CICADA SONGS

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Manuskript nicht eingelangt

ABSTRACT

The song of aggregation produced by the male cicada is very constant and characteristic of the species. A comparison of the songs of cicada from Ceylon and Japan reveals clear similarities between species of the same or related genera. The song is known to be produced by the co-ordinated activity of three sets of muscles, the tymbal muscle supplying the energy for sound production, the tensor muscle straining the tymbal and the abdominal muscles adjusting the size of the tracheal resonator (Pringle 1954). Although a complete analysis has been made in only a few cases (Pringle 1955; Hagiwara & Ogura 1960), it seems likely that the differences between genera stem from qualitative differences in one or more of these muscular systems, and differences between species of a genus mainly from differences in the patterns of nervous excitation of the various muscles. The most important qualitative difference is in the tymbal muscle, which is of the asynchronous (fibrillar) type in *Platypleura*, intermediate in *Meimuna* and synchronous (non-fibrillar) in *Graptopsaltria*, *Tanna* and many other genera including *Magicicada* (Boettiger, 1960).

The songs of 18 species will be presented in the tape recording. The author would be very grateful for copies of further recordings of identified species, in order that the correlation with existing taxonomic schemes may be extended.

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THE ACOUSTIC DETECTION OF BATS BY MOTHS

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The ability of moths to hear the approach of bats has been suspected for nearly a century White, 1877). The extensive studies by Eggers (1919, 1925, 1926, 1928) made it clear that the ympanic organ has an auditory function. Moths of several families were shown to change their ight pattern in the presence of auditory stimuli (Schaller and Timm, 1950; Treat, 1955). An fferent response to sound between 8 kc. and 20 kc. was first demonstrated in the tympanic erve by Haskell and Belton (1956). Treat and Roeder (1956, 1957, 1959) made extensive tudies of the tympanic nerve response of Noctuidae, and occasionally of Arctiidae, Amatidae, ymatophoridae, and Geometridae, to a variety of artificial and natural acoustic stimuli under iboratory and field conditions.

Afferent activity in the tympanic nerve of noctuoid moths can be resolved into impulse equences in three fibers. Two of these (A fibers) are clearly acoustic in function, and heir activity has been traced to the pair of sense cells discovered by Eggers in the scolo- arium of the tympanic organ. The third fiber (B fiber) has characteristics suggesting roprioception (Treat and Roeder, 1959) but its role in behavior is unknown. Since noths fail to react to the usual acoustic stimuli after bilateral destruction of the tympanic coloparia the only afferent pathways over which sound is able to affect their behavior ppears to be the four A fibers. From spike sequences in this small number of nerve ibers it is not difficult to determine the sensory characteristics of the receptors, and by xamining the responses in both tympanic nerves it should be possible to specify the ounds to which a moth is capable of reacting.

In brief, the characteristics of the tympanic organ as revealed by its afferent response re as follows. The two A cells differ in threshold sensitivity by about 20 db. There is no evidence that they are tuned to different frequency bands. They respond to pure ones from as low as 3 kc. in some species to well over 100 kc., showing maximum ensitivity to tones between 15 and 60 kc. Each A fiber responds to a pure tone with a rain of spikes, the initial frequency of which is proportional to sound intensity and may e as high as 1000 impulses per second. The A fibers adapt rapidly to pure tones (50 per ent decline in spike frequency in 0.1 second). Brief sound pulses (1.0 to 10.0 milli- econds in duration) cause an after-discharge in the A fibers that is proportional to ound intensity.

From this it is evident that the noctuoid tympanic organ is well suited for the detection f the short ultrasonic pulses or chirps made by echolocating bats. An initial observation f the tympanic nerve response to cries made by a bat flying in the laboratory led to a eries of field experiments carried out during the summers of 1958 and 1959. A site vas chosen where bats were known to hunt their prey. A table bearing preamplifiers, lectrodes, and dissecting microscope was placed on this spot. Tape recorder, oscillo- cope, and other a. c. operated equipment was placed about 50 feet away in order to ninimize acoustic and electrical interaction.

The effectiveness of the tympanic organ as a bat detector was immediately evident. The approach of a bat was neurally signalled considerably before it became visible in he light of a flood lamp. Short trains of impulses in the A fibres recurred about 10 times second—the approximate repetition rate of the cries made by the cruising bat. As the at approached first one and then both A fibers became active and the number and requency of spikes in each train increased. After a little experience it was not difficult o determine the approach, departure, and other manouevres of a bat by listening to the erve impulse sequence through headphones. By noting the onset of the nerve signal vhile watching the approach of bats at dusk it was found that the tympanic organ could

detect the presence of a bat at a distance of 30 meters or more. This compares favorably with the performance of the best available microphones for the detection of airborne ultrasonics.

Although such simple structures do not appear to be well suited for the precise localization of a sound source it seemed possible that the right and left tympanic organs might respond differentially to an acoustic stimulus and thereby provide information on the direction of its origin. This was investigated monaurally by moving a non-directional source of clicks of constant intensity along eight successive radii chosen at 45 degree intervals around a tympanic nerve preparation. The sound source was brought to that position on each radius where it would produce a standard response, arbitrarily chosen as 1 or 2 impulses per click. Measurements were made only in the horizontal plane with the moth inclined upward at about 30 degrees. A click sounded at a position at right angles to the long axis of the moth produced the standard response on the near side at about twice the distance required to produce the same response on the far side. There seemed to be little difference in sensitivity whether the source was ahead or behind the moth, the responses in both cases being somewhat less than those produced when the source was at the side.

Binaural tympanic nerve activity was recorded in the field as flying bats of at least two species manouevered in the general vicinity of the preparation. Spike sequences in the right and left tympanic nerves were stored on magnetic tape and examined later. When the intensity of the bat cries was not much above the threshold of the preparation a differential response could be detected by inspection of the record and by listening to the spike sequence played back stereophonically. Fewer spikes at a longer latency appeared in the tympanic nerve on the far side. At higher sound intensities (40 or more db above threshold) this differential was not apparent, the nerve responses from both sides becoming saturated and the latencies identical.

The sensory findings described above may define the limits of monaural and binaural sensitivity but tell us nothing about the nature or significance of the behavioral responses. Observation of the manouevres of free-flying moths in the presence of free-flying bats has been in progress for some years (see below), but lack of control over the stimulus source and the circumstances of the contest make it very difficult to define the actions of the contestants. In field work begun during the summer of 1960 electronically generated sound pulses simulating bat cries were directed at free-flying moths. Little can be said at present except that the behavioral responses to these sounds are both striking and various, and show interesting correlations with the sensory data.

Even though details of the evasive movements remain to be worked out there can be little doubt that some moths alter their flight pattern as a bat approaches, while others do not. The survival value of this evasive behavior has been examined by collecting data on the results of encounters between moths and bats under natural conditions. These records, covering the months of June, July and August of 1958 and 1959, are given in Table I. They were obtained in a rural area in Tyringham, Massachusetts.

For many reasons the data require cautious interpretation. Most of the observations were made in the light of a 100-watt incandescent lamp mounted in a photoflood reflector, supplementing the light from a 15-watt GE "black light" (maximum emission at about 3200 Å) which was used as an attractant. The lights were placed on the screened porch of a frame house. They illumined a sector of about 90° looking northward over a lawn partly surrounded by trees. During 1958 both lights were turned off at about midnight each night; during 1959 the black light was left burning until daylight. Use of either light alone did not appreciably affect the character of the results, though total absence of artificial light might well create a very different situation.

The data were derived from bats of at least two and perhaps of three or more species including *Myotis lucifugus* and *Lasiurus borealis.* In dim light it is not possible even for an expert to determine with certainty which species he is watching. Yet the hunting behavior of the different species does differ significantly, so that conclusions which might be valid for the local population of insectivorous bats in general might be grossly in error as regards those of any one species considered separately. *L. borealis* was not recognized among the bats under observation until 1959 when one of four suspected specimens was captured.

Moths of many species are represented. They include predominantly Noctuoidea, Geometroidea and Pyralidoidea ranging in wingspan from about 10 to about 40 millimeters. While moths of these groups are known to be acoustically sensitive, the records also represent in smaller but indeterminate proportion encounters with moths such as Zygaenoidea, Tortricoidea, Bombycoidea, and some of the smaller Spingoidea, none of which has known tympanic organs.

Only those encounters were scored (a) in which both bat and moth were clearly seen before evasion (if any) had begun, and (b) in which the outcome was in no way doubtful. These criteria excluded many encounters which were noticed only when they were already in progress, and many which ended in darkness beyond the lighted area. A moth was scored as a reactor only if its change of flight path was so pronounced as to distinguish it clearly from the usual irregularities in the flight of moths approaching lights. Where this was doubtful the encounter was excluded. Since a near miss might produce atmospheric turbulence which might itself serve as a (non-acoustic) stimulus, encounters were excluded in which the moth appeared to react only on a second or subsequent attack closely following the first approach. Item E_o, "reactions without attack", refers to instances in which the moth made a conspicuous dive as a bat passed above it without making an actual attack.

Reactions were of various patterns, the commonest being an abrupt dive of 1 to 3 meters followed by spiralling or by erratic flight. Often the dive brought the moth into the grass, where it would usually remain for a few minutes. Arctiids and some geometrids seemed particularly inclined to react in this way. Although we noted no systematic relation between the pattern or direction of reaction and the relative positions of the bat and its target, all responses involved a distinct acceleration of flight regardless of direction. The usual distance of the bat from the moth when the reaction began was 1 or 2 meters, the maximum at least 4 meters.

As judged from their frequent dips and swerves the bats (particularly *Myotis*) were feeding not only upon moths but also upon other insects too small for us to see. The red bats (*Lasiurus*) occasionally captured sphingids such as *Paonia myops* with a wingspan of 60 to 70 millimeters. Larger ones were sometimes struck but never captured. Moths of the size range of 10 to 40 millimeters seemed to be preferred, and few of these escaped attack, though many escaped capture.

The red bats attack more swiftly and perhaps more accurately than do the small brown bats, which may account in part for the lower percentage of misses of non-reactors in 1959 (when the red bats were numerous) than in 1958. Another possible reason for the discrepancy between the scores for the two seasons is that the collecting light, which in 1958 had been turned off at the close of each evening's work, was in 1959 left burning all night. This gave the bats a longer time to become familiar with the situation and possibly helped to improve their performance.

Though not a part of the objective record, it is our impression that during the intermittent periods when the bats all go away for a time there are more moths to be seen in flight than when the bats are present. Perhaps there is a general tendency for moths to avoid flight in or into an area where bats are actively hunting. This impression

agrees with the experience of Webb (1953), and is consistent with our own observation of the inhibitory reactions of some moths when confronted at close range with a vocalizing captive bat (Treat, 1955). As shown earlier in this paper, the sensitivity of the tympanic organs would provide and adequate sensory basis for such a tendency.

Table 1

Outcome of encounters between insectivorous bats and medium-sized moths near light

Totals 1958	Totals 1959	Totals 1958+59		Formula (×100)	Percentages 1958	Percentages 1959	Percentages 1958+59
222	186	408	E Scored encounters, all types				
31	31	62	E_o Reactions without attack*	E_o/E	14	17	15
191	155	346	A Attacks, all types	A/E	86	83	85
85	87	172	A_n Attacks on non-reactors	A_n/A	45	56	50
106	68	174	A_r Attacks on reactors	A_r/A	56	44	50
33	61	94	C Catches, all types	C/A	17	39	27
28	54	82	C_n Catches of non-reactors*				
			as % of total catches	C_n/C	74	89	87
			as % of attacks	C_n/A_n	33	62	48
5	7	12	C_r Catches of reactors*				
			as % of total catches	C_r/C	15	11	13
			as % of attacks	C_r/A_r	5	10	7
158	94	252	M Misses, all types	M/A	83	61	73
57	33	90	M_n Misses of non-reactors*				
			as % of total misses	M_n/M	36	35	35
			as % of attacks	M_n/A_n	67	38	52
101	61	162	M_r Misses of reactors*				
			as % of total misses	M_r/M	64	65	64
			as % of attacks	M_r/A_r	95	90	93
			S Selective advantage of reactors over non-reactors	$1-\frac{M_n/A_n}{M_r/A_r}$	29	58	44

* Primary data, i.e., types of encounter actually tallied in the field.

In the Table the raw data on which the various computations are based are indicated by asterisks. They include (1) reactions without attack, E_o, (2) catches of non-reactors, C_n, (3) catches of reactors, C_r, (4) misses of non-reactors, M_n, and (5) misses of reactors, M_r. The Table shows that attacks upon reactors and non-reactors were scored with about equal frequency, but that taking the combined totals for 1958 and 1959, 87 percent of the moths caught in these attacks were those which had failed to react. Of the total attacks on non-reactors, 48 percent resulted in capture, while only 7 percent of the attacks on reactors were similarly successful.

The method of computing selective advantage is similar to that used by Ford (1957). That is, considering reactors and non-reactors as two populations, R and N, a one percent advantage would mean that for every 100 representatives of R which survive to reproduce, only 99 of N would do so. The advantage can be simply computed (as shown in the Table) from the ratio of the percentage of non-reactors surviving attack to that of reactors surviving attack. In considering the high values (29 to 58 percent) obtained by this method it must be remembered that the data are by no means adequate samples of the entire local R and N populations, but represent only those indeterminate but probably small fractions which are actually subjected to direct

attack. Reduced by a factor of ten or even of a hundred, however, the selective advantage of evasion would still be impressive from the evolutionary standpoint. Indeed, one might wonder how moths without means of evasion could have survived at all where predation by bats is a substantial hazard. In this connection it is noteworthy that most moths without known auditory organs are either very large or very small. The exceptions in the size range particularly subject to bat predation include such locally abundant species as the tent caterpillar moths (*Malacosoma* spp.). These may owe their survival though deaf to their high reproductive potential, perhaps aided by their swift and erratic manner of flight.

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THE ACOUSTICAL BEHAVIOR OF CICADES

RICHARD D. ALEXANDER

Manuskript nicht eingelangt

ABSTRACT

The sound-signalling system evolved by the cicadas shows many striking similarities to those known in other insects, and a few distinctive differences. Cicadas are like the Tettingonioidea and Acridioidea in that (1) their auditory organs operate on a similar basis and they utilize the same types of sounds (series of pulses delivered at various rates and in various rhythms) and (2) their sound signals operate in the same general situations and in similar fashions, they differ in being vegetation-inhabiting, diurnal species congregating chiefly through acoustically-orientet flight (no orthopteran possesses this combination of characteristics), in having evidently derived their acoustical system from movements and sounds originally functional in disturbances or during flight (all major orthopteran systems seem derived from courtship activities), and in having evolved more continuously variable adjustments to fluctuations in population size and density than are evident among other sound-communicating insects. The study of acoustical behavior in cicades has been an enigma for two reasons: (1) variability in the behavior of individual species—associated with fluctuations in population size and density—has hindered the development of reasonable hypotheses, and (2) the requirements for successful experimentation—flight space, food availability, specific light intensity, and long-sustained, high-intensity acoustical stimulation—have made it extremely difficult to design controlled tests. The present paper, resulting chiefly from field studies on thirty-odd species of cicadas in eastern North America, is intended to provide insight into these problems and their solution, and to shed light upon the evolutionary pathways followed by cicadas in the elaboration of their sound-communicating system.

PAARUNGSVERHALTEN UND LAUTÄUSSERUNG VON KLEINZIKADEN, DEMONSTRIERT AN BEISPIELEN AUS DER FAMILIE DER DELPHACIDAE (Homoptera-Auchenorrhyncha)

HILDEGARD STRÜBING, Berlin

(Siehe Tafel I—IV)

An Hand eines kurzen Filmstreifens wurde das Paarungsverhalten von drei Delphacidenarten (*Calligypona lugubrina* Boh., *Calligypona adela* Fl. und *Euidella speciosa* Boh.) erläutert. Der Gesang der Männchen wird von paarungsbereiten Weibchen mit rhythmisch artspezifischem Abdomenzittern beantwortet, das akustisch — nach erheblicher Verstärkung — auch für unser Ohr als Trommellaut wahrnehmbar wird. Auch der Gesang der Delphacidenmännchen ist so leise, daß er ohne technische Hilfsmittel für uns nicht hörbar ist. Im Leben der Tiere dienen diese Lautäußerungen als Verständigungsmittel zwischen Männchen und Weibchen, wie bereits früher berichtet wurde (Strübing 1958 und 1959[1]). Diese Lautsignale — oft in Gestalt wohl ausgebildeter Wechselgesänge — leiten die Partner zueinander, wobei die Orientierung aller Wahrscheinlichkeit nach rein akustisch erfolgt. Häufig singt das Männchen, bevor es die Kopulation vollzieht, das Weibchen mehr oder weniger lange intensiv an. Der hierbei auftretende Balz- oder Werbegesang geht aus einer Abwandlung des Normalgesanges hervor.

Am Beispiel von *Euidella* wurde gezeigt, daß Lautäußerungen auch den Charakter von Abwehrsignalen haben können. Immer lehnt das begattete Weibchen nach der Paarung weitere Kopulationen ab und beantwortet den Männchengesang nicht mehr. Stoßen kopulationsbereite oder gar durch Weibchen-Trommeln erregte Männchen während ihrer Suchaktion zufällig auf bereits begattete Weibchen, kann es zu langem Ansingen dieser Weibchen durch die Männchen kommen, wobei die Weibchen durch Schütteln des Körpers, Hinterbein- und Abdomenstoßen, manchmal auch durch Flügelschlagen ihre Ablehnung bekunden.

Bei brachypteren Weibchen ist das Flügelschlagen (zumindest bei den bisher untersuchten 25 Arten) nicht mit einer Tongebung verbunden. Bei makropteren Formen kann in Zusammenhang mit dem Flügelschlag ein Abwehrton ausgestoßen werden. Diese Abwehrlaute sind besonders charakteristisch für abwehrende langflügelige *Euidella*-Weibchen (vgl. Abb. 1) und folgen, begleitet von kurzem Flügelzucken, schnell aufeinander.

Die anschließend demonstrierten Tonbandaufnahmen sollten einen Eindruck von der Verschiedenartigkeit der Delphacidengesänge geben. Darüber hinaus wurde versucht, die Lauttypen phylogenetisch zu deuten und sie in eine anagenetische Stufenfolge zu ordnen.

Ausgehend von der Annahme, daß ein einfacher „Gesang", z. B. ein kurzer Ruf oder ein Schnarrlaut, der ohne Abänderung in mehr oder weniger gleichmäßiger Folge wie ein Signal ausgesandt wird, noch ein recht primitives Merkmal darstellt, wird die Skala der Lautäußerungen im Zuge einer Höherentwicklung immer vielgestaltiger. Es kommt zur Ausbildung gegliederter Strophen und zu einem Werbeverhalten, das schließlich von einem spezifischen Werbegesang begleitet sein kann. Ferner haben höher stehende Arten einen wohl ausgebildeten Wechselgesang, bei

[1] Zoologische Beiträge IV, 1, 1958, Verh. DZG, Münster 1959.

HILDEGARD STRÜBING: Paarungsverhalten und Lautäußerung von Kleinzikaden, demonstriert an Beispielen aus der Familie der *Delphacidae* (*Homoptera-Auchenorrhyncha*)

Abb. 1. Männchen von *Euidella speciosa* balzt begattetes Weibchen an. Zu Beginn 4 Männchen-Rufe, dann folgt auf jeden Männchen-Ruf ein Flügelabwehrschlag des makropteren Weibchens. Im letzten Teil des Oszillogramms werden die Abwehrschläge seltener.

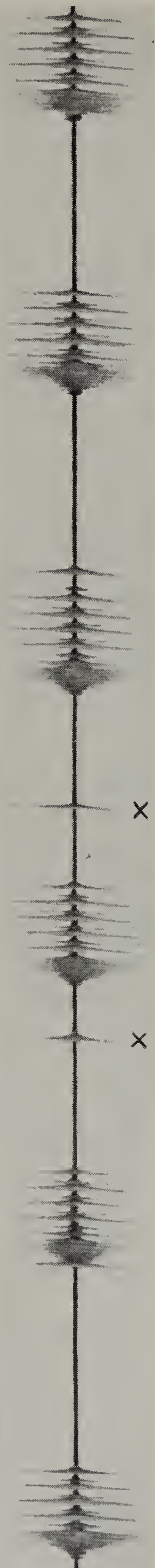


Abb. 2. 1 Männchen, 1 Weibchen von *Conomelus anceps*. Zuerst ein isolierter Männchen-Ruf. Das Weibchen-Klopfen steht z. T. isoliert und ist leicht erkennbar (z. B. an den beiden mit × bezeichneten Stellen), z. T. wird es von dem Männchen-Ruf überlagert. Das Männchen läuft während dieser Lautäußerung suchend umher.

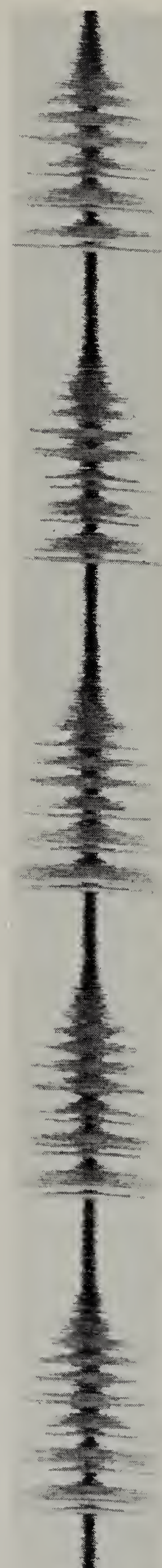


Abb. 3. Grundgesang eines *Stenocranus major*-Männchens. Dieser rhythmische Laut kann lange Zeit in gleichmäßiger Folge wiederholt werden.

HILDEGARD STRÜBING: Paarungsverhalten und Lautäußerung von Kleinzikaden, demonstriert an Beispielen aus der Familie der *Delphacidae* (*Homoptera-Auchenorrhyncha*)

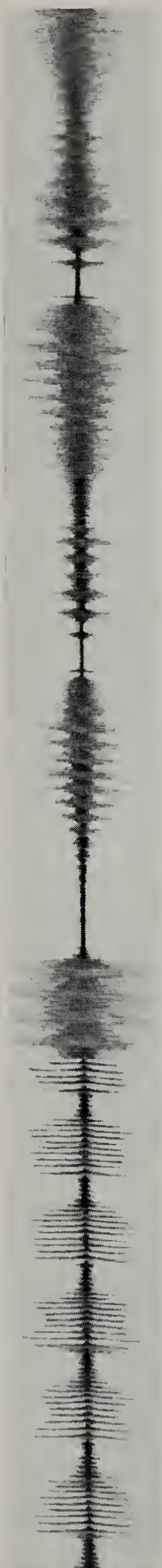


Abb. 4. Dreiteilige Strophe eines Männchens von *Stenocranus minutus*. Etwas mehr links im Bild der sehr kurze Mittelteil der Strophe.

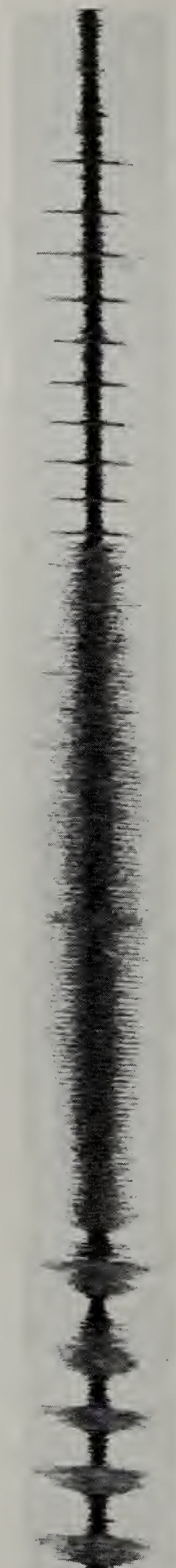


Abb. 6. Wechselgesang von einem Männchen und einem Weibchen von *Calligypona lugubrina*. Weibchen fällt in den Uuuh-Laut des Männchens ein.

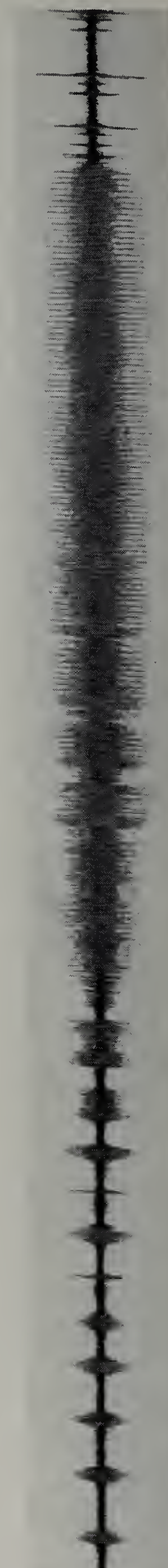
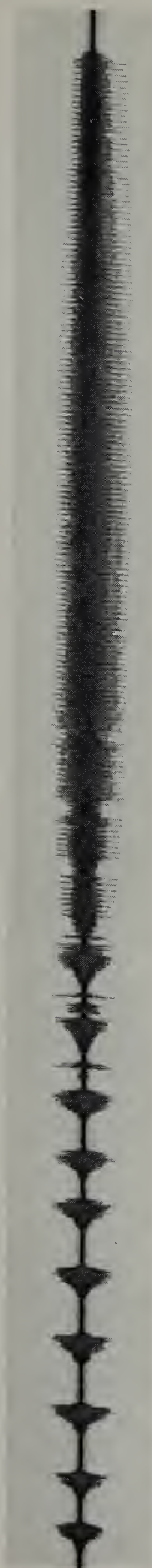


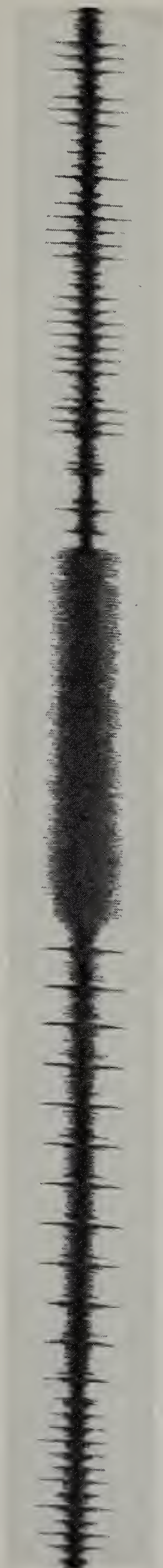
Abb. 7. Werbegesang von *Calligypona lugubrina* mit einem dreimaligen kurzen „Klak“ am Ende der Strophe.

HILDEGARD STRÜBING: Paarungsverhalten und Lautäußerung von Kleinzikaden, demonstriert an Beispielen aus der Familie der *Delphacidae* (*Homoptera-Auchenorrhyncha*)

Abb. 5. Lautmuster jeweils einer Strophe von 6 verschiedenen *Calligypona*-Arten; unter g zum Vergleich *Delphacodes pilosus*.

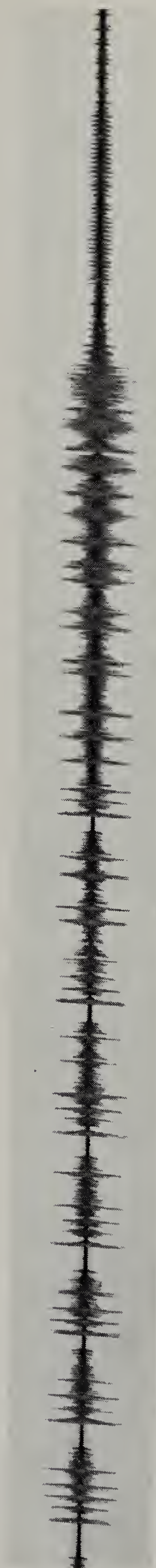


5a) Grundgesang von *Calligypona lugubrina*; ein sich oft wiederholendes Gog-Gog-Gog im ersten Strophenteil, ein langer Uuuh-Laut im zweiten.



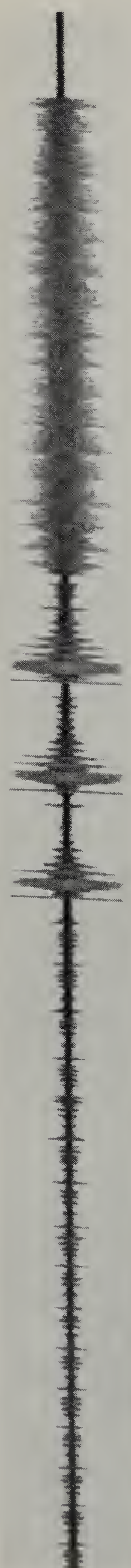
5b) *Calligypona distincta*; ein Schnarren im ersten, ein Uuuh-Laut im zweiten Teil der Strophe.

5c) *Calligypona elegantula*; ein langer Schnarrlaut im Angesang, ein relativ kurzer Ausklang.



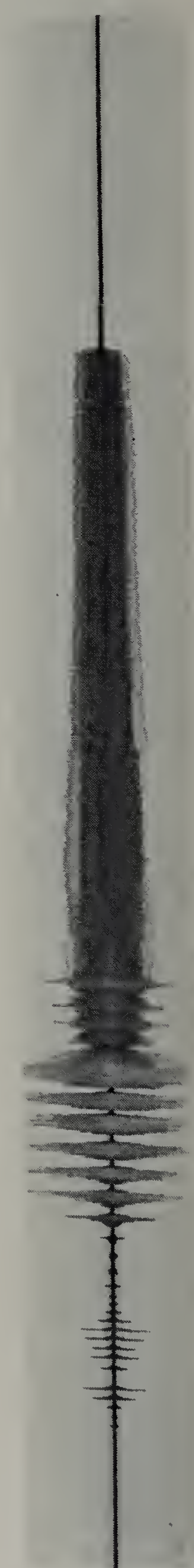
5d) *Calligypona aubei*; ein besonders langes Schnarren und ein sehr leises Ausklingen der Strophe.

HILDEGARD STRÜBING: Paarungsverhalten und Lautäußerung von Kleinzikaden, demonstriert an Beispielen aus der Familie der *Delphacidae* (*Homoptera-Auchenorrhyncha*)



5e) *Calligypona pellucida*; ein metallisch klingender Anschlag und ein vibrierender, rhythmisch gegliederter Ausklang.

5f) *Calligypona dubia*; ein sehr ähnliches Lautmuster wie 5e. Akustisch sind beide Arten kaum voneinander zu unterscheiden, da die Länge des Angesanges sehr variabel ist. In beiden Fällen findet sich ein rhythmisch gestufter Ausklang. Beide Arten sind morphologisch außerordentlich ähnlich (sibling species im Sinne von Kontkanen. Arch. Soc. 'Vanamo', Helsinki 1953).



5g) *Delphacodes pilosus*; der Angesang hat den Charakter eines kurzen Schreies, der im 2. Teil der Strophe ausklingt.

dem das Weibchen an einer ganz bestimmten Stelle in den Männchengesang einfällt. Als Beispiel einer primitiven Lautäußerung wurde *Conomelus anceps* Germ. gewählt. Das Männchen besitzt nur einen kurzen Ruf, mit dem es, im Falle einer Antwort eines Weibchens, in sein monotones und lang anhaltendes Klopfen einfällt. Da beide Signale in einem unterschiedlichen Rhythmus erfolgen, können sich die Lautmuster (vgl. Abb. 2) z. T. überlagern, z. T. sind sie getrennt wahrnehmbar. Ein alternierender Gesang mit gegenseitigem Rufen und Antworten ist „noch" nicht ausgebildet. Auch *Stenocranus major*-Männchen haben einen einförmigen Grundgesang (Abb. 3), kein spezifisches Balzverhalten und keinen Werbegesang. Hat ein Männchen ein Weibchen gefunden, kommt es zu mehr oder weniger langem Ansingen, bei dem lediglich der Grundgesang intensiviert wird, bevor das Männchen die Kopulation vollzieht.

Stenocranus minutus F. ist eine höher entwickelte Art und besitzt eine wohl ausgebildete dreiteilige Strophe (Abb. 4). Der Mittelteil der Strophe ist sehr kurz und wird in Zusammenhang mit einem kurzen Flügelschlag im Balzgesang verstärkt, d. h. ist dann besonders laut. Vor- und Nachgesang können in ihrer Länge individuell und auch bei ein und demselben Tier stark variiert werden.

Von den 4 deutschen *Stenocranus*-Arten haben *Stenocranus fuscovittatus* Stål und *Stenocranus longipennis* Curt. zweifellos den höchstentwickelten Gesang. Die lange Strophe ist außerordentlich variabel, ihre Teile können beliebig aneinander gereiht werden, und es ist ein typischer Balzgesang ausgebildet. Dabei sind in Zusammenhang mit mehrfach erfolgenden sehr raschen Flügelschlägen intensive Klopftöne in den Gesang eingebaut. Die Variabilität des Gesanges wird in einer zusammenfassenden Bearbeitung über Lautäußerung und Paarungsverhalten der Delphaciden gezeigt werden.

Abbildung 5 veranschaulicht das Lautmuster von 6 verschiedenen *Calligypona*-Arten. Alle haben eine zweiteilige Strophe: einen mehr oder weniger langen Angesang und einen lang gezogenen Ausklang, der ein singendes Geräusch oder auch ein gedehnter Uuuuh- oder Öööök-Laut sein kann. Bei den am höchsten entwickelten Arten fällt das Weibchen an einer ganz bestimmten Stelle in den Männchengesang ein, das Männchen schweigt darauf, so daß es zu einem präzisen Wechselgesang, zu einem Rufen und Antworten kommt, vgl. Abb. 6. Der Balzlaut wird bei den *Calligypona*-Arten, sofern ein Werbegesang vorhanden ist, an die Strophe, d. h. an den Ausklang angehängt. Bei *Calligypona lugubrina* ist er ein kurzes „Klak-Klak", begleitet von intensivem Flügelschlagen (Abb. 7). Dabei ist der Flügelschlag für die Entstehung des Tones unwichtig: Männchen, denen man die Flügel entfernt hat, erzeugen während ihres Balzverhaltens den gleichen Laut.

Die systematische Stellung der Gattung *Delphacodes* Fieber war früher recht umstritten. Morphologische Merkmale hatten Haupt veranlaßt, sie in die Verwandtschaft der Stenocraniiden („Megamelinae" nach Haupt in Brohmer: Tierwelt Mitteleuropas, 1935) zu stellen, während in letzter Zeit die Ansichten sich mehren, *Delphacodes* sei mit den Calligyponen näher verwandt. Auf Grund der akustischen Phänomene fällt die Entscheidung nicht schwer (vgl. Abb. 5g). Die Ähnlichkeit im Aufbau der Strophe mit der der Calligyponen ist frappierend, doch wird der Werbelaut nicht an die Strophe angehängt wie bei diesen, sondern in den Gesang eingebaut.

Die in den Abbildungen 1—7 dargestellten Lautmuster wurden mit einer Geschwindigkeit von 5 cm pro Sekunde aufgenommen. Die Amplituden wurden, den photographischen Zwecken entsprechend, am Oszillographen eingestellt und gestatten keinen Lautstärkevergleich zwischen den einzelnen Arten. Die Tonbandaufnahmen wurden durch einen Hoch- und Tiefpaß gefiltert, so daß die abgebildeten Oszillogramme in einem

Frequenzbereich zwischen 300 und 3500 Hz, dem Schwerpunkt der Lautäußerungen der untersuchten Delphacidenarten, gewonnen wurden.

Weitere Beispiele und Einzelheiten werden in der oben erwähnten zusammenfassenden Darstellung über die biologische Bedeutung der Lautäußerungen von Delphaciden dargelegt werden, in der auch die Versuchsmethodik mit ihren technischen Daten zur Aufnahme so geringer Lautstärken ausführlich beschrieben werden soll.

THE ELECTROPHYSIOLOGY OF PHONORECEPTION IN MOSQUITOES AND OTHER INSECTS

M. L. WOLBARSHT

Manuskript und Abstract nicht eingelangt

Lichtbildervortrag A. FABER

GRUNDFRAGEN EINER VERGLEICHENDEN BEHANDLUNG DER BIOAKUSTISCHEN ERSCHEINUNGEN BEI INSEKTEN

siehe in Mitt. staatl. Mus., Stuttgart 1962

SYMPOSIUM II

LONG-RANGE DISPLACEMENTS AND MIGRATION OF FLYING INSECTS

WIND AND ORIENTATION OF MIGRATING BUTTERFLIES IN COMPARISON WITH BIRDS

D. A. VLEUGEL, The Hague, Netherlands

In the Swiss ornithological journal "Der Ornithologische Beobachter" of 1952 I gave instances of migrating Chaffinches (*Fringilla coelebs*) altering their direction in conformity with an alteration in the direction of the wind.

The hypothesis was advanced that they take their initial direction in early morning from the sun, and can keep this direction by subsequently orientating themselves to the wind, as long as the wind direction remains unaltered. It was suggested that this hypothesis accounts for the lack of migration on days with no wind or variable winds.

Now the question is: How is this in migrating butterflies? My experience is that butterflies migrate with winds from different quarters, as is well-known from the literature. However, I have no experience, whether butterflies migrate in a calm and with changing winds. It was necessary to search for observations of this kind in the literature. I know from an experience of many years in bird migration that it is better not to use the observations of migrating butterflies which are following coast lines and the like. For this reason the fine observations of Blunck (1954) made on the coast of the Baltic, could not be used. We used only the observations on migrating butterflies flying on a broad front.

Let us consider the different types of weather.

1. With no wind.

Williams (1958, p. 117—123) does not mention any observations of migrating butterflies with a calm, although he gives summaries of more than thousand observations on several species. Nor does Lempke (1957, p. 66—67) mention such cases in 11 observations of *Pieris brassicae* and *rapae*. Blunck mentions Large Cabbage Whites migrating in a calm, but this was along a coast. Geoffrey Beall mentions also an observation of a slight migration of Monarch Butterflies in Florida with a calm, but this was again along a coast line (Dec. 10th 1951).

2. With variable winds.

There have been made some observations on migrating butterflies under these circumstances, one by Burton & Owen (1954) and one or two by Blunck (1954). However, in all cases this regards migration along a leading line, so that we cannot

use them. Williams (1958) and Lempke (1957) do not mention any migration with variable winds. Beall (1941, p. 127) writes: "Twice at Point Pelee the normal progression, with the advance of the day, in migration, seemed to be interrupted by a change of wind."

3. With altering winds.

It will be clear that as a rule a change in the direction of the wind which is only slow, will remain unnoticed by migrating butterflies. However, when the direction of the wind alters quickly, it will be discovered by the migrants and they will stop their migration. For this reason it is difficult to make observations on migrating butterflies altering their direction in conformity with an alteration in the direction of the wind. Even in birds which migrate so much more, it is not easy to discover such alterations in the direction of their migrations. For this reason it was surprising to me to find two cases of butterflies altering their direction in conformity with the wind.

a. Williams (1958, p. 114) writes: ". . . at Gaza in Palestine at the end of April 1917 Pendlebury observed Painted Ladies flying towards the north-west in the morning, and in smaller numbers to the south-east in the evening. These changes were associated with, and possibily related to, changes in the wind — from a sea breeze from the north-west in the morning to a land breeze from the south-east in the evening. In each case the butterflies were flying against the wind." In one other case (Williams, p. 114) no direction of the wind is mentioned.

b. According to Blunck (1954, p. 498—499), there were alterations either in the direction or in the number of the migrating butterflies connected in some way with the direction of the wind. At any rate when there was migration with variable winds, it was only small. However, Bluncks observations were made on a coast where the direction of migration was more or less determined by the direction of it, so that these observations are of little value for our purpose. Besides, as I know from migrating birds, the situation on coasts is very difficult to understand, so that we better leave out Bluncks records. However, he mentions one case of migrating Large Cabbage Whites migrating on a broad front. There was a change in direction in accordance with an alteration in the direction of the wind. Blunck (op. cit., p. 498—499) writes: "So berichtete der Fischer Wellendorf in Strande an der Förde, daß am 2. August bei unvermittelt sonnigem Wetter von 1000—1300 oder gar 1400 bei zunächst leichtem Südwind (Stärke 2), der mittags stärker werdend (Stärke 2—3) nach Südwesten und Westen drehte, zwischen der Bülker Leuchtboje und dem 5 km vom Strand entfernten Feuerschiff Kiel viele Kohlweißlinge dicht über dem Meeresspiegel in Richtung Nordost geflogen seien. Die Flugrichtung ist später von Nordost nach Südost umgeschlagen."

Is initial orientation by the sun possible for migrating butterflies?

It is generally believed now that the sun is the governing factor in the orientation of migrating birds. Vleugel (1953) has summarised several reasons for this. It has even been proved for Starlings in the laboratory (Kramer 1952).

In my opinion it could also be the governing factor in the migration of butterflies. They would only have to choose a horizontal angle with the direction of the sun in the morning as is assumed that birds do. According to Beall (1941) and Blunck (1954) migrating butterflies as a rule start their journey early in the morning. Besides, butterflies start their migration chiefly when the sun is shining.

I agree with Williams (1958, p. 126) that it seems difficult to believe that migrating butterflies allow for the change of angle necessitated by the apparent movement of the sun. It has been proven for Starlings in the laboratory by Kramer (1952). And

L. Tinbergen (1956) found that Chaffinches improve their initial orientation, when after some overcast days without any visible sun the sun is only visible during a short period in the afternoon. But for actually migrating birds it has not been proven so far. As regards insects, bees are the only insects of which is more or less proven that they have an internal clock and an inherited knowledge of the direction of the movement of the sun (summary in Baerends, 1959). In my opinion the possibility exists that migrating butterflies allow for the change of angle necessitated by the apparent movement of the sun. But it will be more difficult to prove it with certainty than in actually migrating birds. There are two observations of Monarchs resuming their migration after an interruption by an alteration in the direction of the wind (Beall 1941, p. 127). He says: "First, on September 13, 1935. The light southwest wind, which had previously prevailed, turned about 3.00 p. m. to the northeast. The flight was renewed then. Second, on one day, September 22, 1935, an observer saw a flight out over the land in the afternoon and he recorded that on that day a west wind of the morning changed to a north wind in the afternoon." In both cases it is probable that the Monarchs stopped their migration because of a turning wind, but resumed their migration after the wind had become steady again. Then it would be like it is in birds. Birds generally seem to find it too difficult to use the moving sun as a point of reference during their migration and therefore use wind direction. Apart from this wind orientation seems to be more accurate.

The apparent method to use the direction of the wind to maintain a straight course.

A butterfly flying in a constant wind would not be conscious of the air movement. It cannot know the direction of the wind, unless the wind is changing strength. How could it fly then a constant angle with the direction of the wind? After a study of years I think that I have found the solution of this problem. In a recent paper I (1959) have explained how a bird (and a butterfly) could do it. Because I have not sufficiently studied English terminology of dynamics and air navigation I will borrow from my German-written paper. Besides I had to introduce a new term for which I do not know a good English equivalent at present. On p. 83 of this paper I say as follows:

„Wenn ein Zugvogel, z. B. ein Buchfink, der sich auf dem Zug befindet, mit Hilfe eines konstanten Winkels auf die Richtung der (z. B. aufgehenden) Sonne seine Normalzugrichtung bestimmt hat, so geschieht es nur bei Windstille oder bei reinem Mit- oder Gegenwind, daß seine Kopf-(Schnabel-)Richtung mit seiner Zugrichtung zusammenfällt. Bei allen anderen Windrichtungen hat der Vogel einen Winkel zu formen zwischen seiner Kopf-(Schnabel-)Richtung und seiner Zugrichtung. Dieser Winkel wird um so größer, je mehr der Wind von der Seite kommt. Es ist notwendig, hier ein Beispiel zu geben, und wir wählen wieder den Buchfinken.

Wie man aus der Figur sieht, ziehen die Buchfinken ungefähr in Südwestrichtung (= Richtung der Linie AZ). Sie haben dazu ihren Kopf in die Richtung der Strecke AF zu stellen und in diese Richtung (in Beziehung zur Luft) zu fliegen. Vom Westwind der genannten Stärke seitwärts verdriftet, ziehen sie dann ungefähr in SW-Richtung in Beziehung zur Erde.

Es ist die Frage, ob die Buchfinken den Winkel FAZ in der Tat bilden können. Sie haben dazu die beiden Strecken AF und AZ auf dem Boden abzulesen. Dies geschieht unmittelbar, wenn sie ihren konstanten Winkel in Beziehung zur Sonne (wahrscheinlich bei Sonnenaufgang) bilden. Sie sehen dann genau, wie sie den Kopf zu stellen haben, d. h. in welche Richtung in Beziehung zur Luft sie zu fliegen haben, um in der zuständigen Richtung in Beziehung zur Sonne zu fliegen. Wenn die Buchfinken nun weiter darauf achten, daß der Winkel FAZ konstant bleibt, so ziehen sie (wenn Windstärke und Windrichtung unverändert bleiben) stets gut richtungstreu. Nach einiger Zeit werden sie bemerken, daß der Winkel FAZ z. B. zu groß wird. Sie haben dann ihre Kopfrichtung so lange zu verlegen, bis der Winkel wieder gleich groß wie im Anfang wird, und ihre Zugrichtung wird wieder SW.

Die Kopf-(Flug-)Richtung AF können die Buchfinken dadurch feststellen, daß sie sehen, wohin der Schnabel weist. Die Zugrichtung AZ ist die Linie in der Landschaft, die sich für den Blick des Vogels in Ruhe befindet. Man kann auch sagen: Es ist die Linie in der Landschaft, wo keine parallaktische Bewegung stattfindet.

Es ist weiter notwendig, daß im gegebenen Beispiel die Buchfinken darauf achten, daß der Wind immer von rechts vorne kommt. Wie gesagt, können sie ja Gegenwind, wenn er schnell anschwillt, sehr kurz mit dem Tastsinn empfinden. Sie können dasselbe auch sehen, d. h. die Kopfrichtung muß immer nach rechts von der Zugrichtung weisen.

Nennen wir den Winkel FAZ den Abweichungswinkel, weil er angibt, wieweit die Kopfrichtung (= Flugrichtung in Beziehung zur Luft) von der Zugrichtung abweichen muß, um eine Verdriftung durch einen Wind von gewisser Stärke und Richtung zu neutralisieren.

Sogar wenn die Vögel ihre Zugrichtung zeitweise ganz und gar verloren haben, können sie diese wieder zurückfinden und sind also unabhängig von der Richtung der Sonne geworden. Sie brauchen nur so lange zu suchen, bis der Winkel FAZ wieder der gleiche wird wie vorher und der Wind von rechts vorne kommt. Wie ich früher (1952) bemerkt habe, wird nicht gezogen, wenn der Wind veränderlich ist. Inwieweit schon ziehende Vögel Änderungen der Windrichtung bemerken, können wir erst nach vielen Beobachtungen am reinen Breitfrontzug sagen. Jedenfalls dreht der Zug bisweilen mit einem drehenden Winde mit (l.c.).

Es wird klar sein, daß für andere Windrichtungen und Windstärken die Abweichungswinkel anders werden. Es gibt hier sehr viele mögliche Fälle. Gerade weil dies so ist, ist diese „Projektionsmethode" für die Zugvögel brauchbar. Natürlich gibt es auch bei dieser Methode gewisse Fehlerquellen. Wir wollen diese hier noch nicht besprechen, weil es besser ist, sie erst genauer zu untersuchen. Meines Erachtens sind die Fehlerquellen aber nicht so groß wie bei der Umrechnungsmethode (bei Sonnenschein), verbunden mit Visier- oder Parallaxemethode (Vleugel 1955) bei bedecktem Himmel. Dadurch ist die Projektionsmethode wahrscheinlich die normale sekundäre Orientierungsweise geworden."

That migrating butterflies really fly an angle between the direction of their migration and the body-axis is reported by Williams (1958, p. 124). He quotes there Gleason who wrote: "When the wind was at a maximum from the south, and the insects (an unidentified Skipper butterfly in New Mexico) were travelling straight across it, they faced towards the south-east as if to avoid being blown to the north of the point that they wanted to reach."

Blunck (1954, p. 490) also says: "In der Tat war beim Wanderflug von *Pieris brassicae* deutlich sichtbar, daß die Körperlängsachse je nach Stärke des Windes auf diesen mehr oder minder stark zu bzw. von ihm weg gerichtet gehalten wird."

With the type of wind orientation I mentioned, it is of course necessary for the butterflies to have a clear view of the country they are crossing. Williams (1958, p. 109—110) mentions explicitly that migrating butterflies prefer to fly lowly. He says:

"When migrating in open forest, butterflies fly under and between the trees; but when the forest is dense they will fly above the canopy at about the same height above this that they would have been above the ground. When they come to a clearing in the forest they do not cross it at tree-top level, but flutter down the near side and across the bottom, and they rise again almost vertically on the opposite side. The same behaviour has been noted in California when migrating butterflies were crossing narrow canyons, they flew down into the gully and across the bottom."

A great advantage of the type of wind orientation I mentioned is for migrating butterflies that they can use the same type when crossing the sea. In this case they can do it with the help of the wind lanes. That they fly in a steady direction I have seen several times in the province of Zeeland in The Netherlands, when migrating *Pieris* species were crossing the Scheldt. I saw it also of *Colias* and other species. Another observation of mine is mentioned by Lempke (1948, p. 306). On Aug. 23rd 1947 I saw numbers of *Pieris*, mostly *rapae*, fly in a western direction on the north coast of Walcheren. They were flying above the mouth of the Easter Scheldt and went into the North Sea. On the same day Dannreuter mentions that great swarms of *rapae* flew into England from the North Sea. This proves that they maintained direction across the North Sea.

Williams (1958, p. 111) says about this question: "To show that the sense of direction is as good over water as over land, I can quote, from my own observation, the case of several dozen Painted Ladies flying steadily to the north over the Mediterranean in July, 1921."

Orientation under overcast sky and at night.

Harz & Wittstadt (1957, p. 31) and Williams (1958, p. 130) state, that butterflies also migrate under overcast skies. Also is reported (Harz & Wittstadt, p. 31 and Williams l. c., p. 105 and 169) that migration takes place by night. In this cases it will be clear that navigation by means of the direction of the wind is possible too. Let us end by giving only some short remarks on migration under an overcast and by night separately.

1. Under an overcast. Several ornithologists have made the suggestion that migrating birds choose aiming-points to maintain a straight course, when there is no sun. I rejected this means of navigation to be used for longer stretches on several grounds. I think that my arguments can be used as regards this view for migrating butterflies too. I will refrain from giving them here and refer to my paper (1955).

By night. In this case navigation by means of the direction of the wind is possible too. It has been made probable by me (1954) that this type of navigation is used by birds, at least thrushes. Probably another type of wind orientation is used by birds migrating during the night, because the type I mentioned in this paper is thought to be then very difficult. But flying birds and butterflies will be able to assess the direction of the wind by means of its changing strength.

Whether butterflies orientate themselves by means of the stars as birds can do (Sauer, 1955), I do not know. At the present state of knowledge it would be unwise, to give an opinion now. Neither would I reject the possibility that the moon is used, as Harz & Wittstadt (1957, p. 31) do, because butterflies also migrate when the moon is not there.

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ÜBER EINEN FALL VON ANEMOCHORER AUSBREITUNG DER BLATTLÄUSE IN FINNLAND IM SOMMER 1959

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Im Jahre 1959 kamen viele Schädlingsarten und besonders einige Blattlausarten außergewöhnlich reichlich in Finnland ebenso wie in vielen anderen Teilen Europas vor. Dann war das beinahe den ganzen Sommer geherrschte warme und wenig regnerische Wetter klar vorteilhaft der Vermehrung der Blattläuse wie bekannt. Dadurch wurde der einheimische Bestand kräftig vermehrt. Es ist auch offenbar, daß der einheimische Bestand der meisten Arten genügend war, um die nötige Abgangspopulation einer großen Massenhervortretung zu geben. Einige Arten erhielten doch offenbar einen merkbaren Zuschlag außerhalb der Grenzen des Landes von einem von dem Wind beförderten fremden Bestand, wie man in einem Falle Schlüsse ziehen konnte. Eine solche Art war *Euceraphis punctipennis.*

In den ersten Tagen des Juni (2.—5. 6. 1959) stellten die Verkehrsflieger fest, daß besonders in den südlichen und mittleren Teilen Finnlands (auf den Flugrouten Helsinki—Jyväskylä—Kuopio—Kajaani und Kuopio—Joensuu) es an einigen Tagen beinahe während der ganzen Flugetappe (etwa 600 km) in der Luft, auf der Flughöhe (die hauptsächlich bei 1200—1300 m, aber in gewissen Fällen bei 2000—2500 m lag) sehr reichlich kleine Insekten gab, die massenweise gegen die Gläser der Führergondel, zermalmt, die Sichtbarkeit belästigten. Die Dichtheit der Insekten in der Luft war so groß, daß sie deutlich wie ein Schwarm sichtbar waren. Die von dem Flugzeug an einem Tag erhaltene kleine Probe enthielt zu einem ansehnlichen Teil Reste der *Euceraphis punctipennis* und reichlich Teile des Körpers irgendeiner Mückenart.

Bei der Forschung von meteorologischen Karten und Wetterbeobachtungen in der erwähnten Zeit konnte folgendes festgestellt werden. Seit dem Beginn des Juni waren die herrschenden Luftströmungen solche, daß z. B. aus Baltikum und mittleren Rußland und von der Richtung von Polen Insekten sehr gut hierhertreiben konnten. Z. B. 1—5/6 waren die thermischen Steigungsströmungen allgemein auf weiten Gebieten südlich und südöstlich von Finnland. Sie konnten die Blattläuse und andere Insekten in die höheren Luftschichten heben, und dort richteten sich die Luftströmungen in jener Zeit hauptsächlich gegen Finnland, wo mittelmäßige oder schwache Süd- und Südostwinde (besonders in den größeren 1400—3000 m-Höhen, und oft auch in den Oberflächenschichten) herrschten. — Eingehendere Untersuchungen zur Erörterung des Phänomens wurden nicht ausgeführt. Doch erscheint es offenbar, daß in unser Land dann mehrere Schädlingsarten in so reichen Mengen hierhergetrieben sein würden, daß sie außergewöhnlich große Schäden zustande brachten. U. a. zweifle ich, daß die Blattlausart *Rhopalosiphon padi* L. dann von außerhalb der Grenzen des Landes kam. Diese Art verursachte in Finnland sehr große Schäden.

Eine entsprechende anemochore Treibung eines Schädlings wurde auch im Jahre 1958 bemerkt, da die Kohlschabe (*Plutella maculipennis* Curt.) durchwegs in ganz Finnland als ein sehr belästigender Schädling hervortrat. Imagines dieser Art erschienen plötzlich am Anfang des Juni und auch am Ende des Juli in so großen Mengen, daß sie nicht aus der in Finnland bemerkten Menge von Jugendstadien sich hatten entwickeln können. Weil Imagines dann außerdem auf weiten Sümpfen und Wäldern und in großen Mengen auch auf den kleinen ganz pflanzenlosen Felseninseln und Schären der Südküste Finnlands getroffen wurden, bewies dieses unbestreitbar, daß die Treibung der Art aus ferneren Gegenden stattfand. In den gegebenen Zeiten herrschten beinahe

V. V. YAKHONTOV: Seasonal migrations of lady-birds *Brumus octosignatus* Gebl. and *Semiadalia undecimnotata* Schneid in Central Asia

Fig. 1. Artifical gathering place for wintering of Brumus octosignatus Gebl.

Fig. 2. Artifical gathering place for wintering of Semiadalia undecimnotata Schneid.

in einer Reihe die S-SO-O-Winde (Geschwindigkeit hauptsächlich 2—6 m/sek.), die offenbar Kohlschabenimagines von dem Gebiete der Sowjetunion brachten (Kanervo 1960). Eine ähnliche Wanderung ist auch in vielen anderen Fällen (Ormerod 1892, Hardy 1938, Harcourt 1957, Williams 1958, festgestellt worden.

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SEASONAL MIGRATIONS OF LADY-BIRDS BRUMUS OCTOSIGNATUS GEBL. AND SEMIADALIA UNDECIMNOTATA SCHNEID IN CENTRAL ASIA

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(See plate V)

The lady-birds *Brumus octosignatus* Gebl. and *Semiadalia undecimnotata* Schneid are widely distributed in Central Asia and have here regular seasonal migrations. They hibernate in colossal accumulations on peaks of low mountain ridges; the *Brumus* likewise hibernates in windward tops of hills. The *Brumus* hibernates at altitudes from 400 to 2,500 metres above Sea Level, the *Semiadalia* — at altitudes of 2,000 metres and higher. The *Brumus* gather at the base of shrubs and grassy growths, the *Semiadalia* — between stones and in the cracks of rocks. The discovery of abundant accumulations permitted us to carry out observations over scores of assembles of both species. On ridges at altitudes from 2,000 to 2,500 metres the *Brumus* begin to gather about the 20th of July, at altitudes 1,500 to 1,600 metres — during the first ten days of August and at altitudes from 400 to 600 metres — from the middle of August. The gathering of *Semiadalia* at altitudes characteristic for this species occurs approximately five days earlier than the *Brumus*. The gathering at once becomes of a mass character. Intensive gatherings continue on separate ridges for three days and then gradually slackens off and after two weeks only individual beetles flying in from the valleys are observed. (The gathering begins about eight o'clock in the morning and ends by six-seven o'clock in the evening.) The temperature and humidity does not influence directly the gathering of lady-birds in the winter gathering places. We observed the gathering at 19 to 94 per cent. relative humidity and at temperatures from 20 to 36° C.

The time of the gathering for wintering depends on the time the beetles hatch from the pupae of the last generation. On mountainous slopes at altitudes from 1,100 to 1,500 metres the *Brumus* developes in one generation during the summer but on the

plains of Northern Uzbekistan and South Kazahkstan — two generations. The critical lower barrier to the development of the *Brumus* is the temperature at 20° C., (the higher barrier for the development of this species is the temperature at approximately 36° C.). The lower barrier for the development of the *Semiadalia* is equal on the average to 13.6° C. Beetles gather for the winter without nourishment with ample quantities of fat in their bodies.

The beetles prefer to settle down in old gathering places, this permits us to map out the gathering places. In the old gathering place of many year's standing, the beetles are attracted not only by the convenient location of the place but also by the smell of the old bodies of lady-birds. However, the smell of beetles, killed by the fungus *Tarichium*, frightens them off. The amount of insects in separate gatherings of both species is extremely different: from a few score of specimens to several hundred thousand specimens in one place. The more ordinary places have several thousand and tens of thousand of specimens. Both species of lady-birds studied—sit during the cold periods—at temperatures lower than 13° C., in dense masses. On being disturbed, already at a temperature of 12° C., they begin to move their antennae, legs and even crawl, but, later they again crowd into dense masses. Places suitable for the lady-birds to hibernate are so specific that the author and his assistants could without climbing from the foot of the hills and mountains determine the presence of the beetles' winter gathering places on them. The conditions necessary for the gathering of the lady-birds for winter were checked by creating artificial gathering places and placing them on watersheds of not big mountain ranges, which may be seen from the photographs—for the Semiadalia heaps of stone, for the *Brumus*—well ventilated boxes with wormwood bushes and a handful of old bodies of beetles. A great amount of beetles accumulated in the artificial gathering places.

The dispersal flight of the beetles in spring from gathering places lying at lower levels occurs earlier than that from the more elevated locations. It has been proved by observations with the help of thermometers, thermographs and psychrometers as well as by the dissections of the beetle's sexual system, that temperature and humidity have no direct influence on the flying away. They influence only indirectly, determining the time of the development of the sexual products and the beetles with the initial stage of the development of ovaries and testes fly away but earlier than this stage they do not fly away. We believe, that here must be place for incretory action. While the gathering of beetles takes place promptly and extremely intensive in Autumn, the dispersal flight in Spring is very prolonged in time and prolixity, it is more considerable in the *Semiadalia* than in the *Brumus*. From the biological point of view, the dispersal flight is co-ordinated with the time of the appearance of aphides in the valleys. (The more common food [in spring] of both species of lady-birds in Central Asia is the aphid *Macrosiphum jaceae* L.) After the dispersal flight and up to the moment of egglaying the *Brumus* requires food in the course of fifteen days on the average and the *Semiadalia*—eleven days. The dispersal flight from mountain ranges, thanks to the different altitudes of winter gathering places, go parallel with the amount of the aphides in the valleys. From the more lower gathering places the dispersal flight of the lady-birds ends in the middle and in the third decade of April. From the most elevated locations the beetles end their dispersal flight towards the end of May. In the warm early spring time some large accumulations of *Brumus octosignatus* may break-up into much smaller groups

The data received by us on the biology of the *Brumus octosignatus* and *Semiadalia undecimnotata* allows us in the following manner to interpret from the general biological point of view the reasons for their seasonal migrations. The active life of these lady-birds is closely connected with periods of propagation of the aphides in Central Asia, living on xerophilous vegetation. In the period of hatching from the pupae of the second

generation of lady-birds the plant vegetation ends in the steppes of Central Asia and South Kazahkstan and the amount of aphides reduces to a minimum. No reserves of food remain for the lady-birds, although the temperature is favourable for their active life. The instinct (evidence from extremely ancient times and in a long line of generations) in the lady-birds was worked out for flying away to cooler places where the beetles loose their activity. And the beetles which did not fly away had to perish from starvation by not leaving their offspring. When the larvae feeds abundantly, the beetles of the second generation hatching from the pupae possess such an amount of fat that they may endure a prolonged hibernation right up to the time of the mass appearance of the aphides in spring. Similar accumulation of fat occurs with mammals hibernating in not an active state. The more cooler locations are the peaks of mountains which are open to the winds. The smell of beetles which died in the last wintering in the gathering places indicates to the lady-birds a more convenient place for sleeping and settling in masses, provides a better chance for survival at very low temperatures. That is why the gathering at much higher summits occurs earlier and at much lower summits later. It is possible to assume that the earlier the beetles of the second generation hatch from the pupae they migrate to greater heights.

Sufficiently high temperatures for the activity of the beetles in the mountains where occur winter accumulations of lady-birds come much later than in the valleys. Up to the time of the beetle's flight to the valley there is already active parallel propogation of aphides on rapidly vegetating plants. The beginning of the maturity of the sex glands, evidently, create in the lady-birds a stimulus to the increase of the instinct of flying away. At sufficiently high temperatures in the wintering places the beetles begin to develop sexual products. Yellowness appears in the testes of the males, there is a formation of the lower egg cell in the ovarian tubes of the females and before the dispersal flight there even occurs coupling. The complete development of the egg requires feeding, but at this time there is not enough food available for the beetles in the mountains. The unequal warming up of the lady-birds in various layers of the accumulations conditions non-simultaneous initial stages of the development of the sexual products. Here, the dispersal flight occurs even in the gathering place, located on identical altitudes above Sea Level, gradually, parallel with the growing amount of aphides in the valleys.

From this it becomes clear in the need for the *Semiadalia* to winter at much higher places than that which is possible for the *Brumus*. The thing is that the development of the sexual products in the ovaries, the embryonic and postembryonic development in the *Semiadalia* occurs considerable quicker than in the *Brumus*, regarding this we have quite detailed data, which were received at different conditions of temperatures and humidity. Under similar early dispersal flights of the *Semiadalia*, as this takes place from the lower gathering places of the *Brumus*, the *Semiadalia* would have laid eggs so early that her larvae would not have been provided with food.

The stimulus for the transmigration of the lady-bird species studied as well as the time and place for the transmigration and connected with these accumulations of specific quantities of fat masses in the beetles' bodies of the second generation, which we likewise studied, were created, thanks to the insufficiency of food for the lady-birds, beginning with the second half of the summer right up to the spring of the following year.

AIR DISPERSAL OF INSECTS IN THE PACIFIC AND ANTARCTIC AREAS

J. LINSLEY GRESSITT

Manuskript nicht eingelangt.

ABSTRACT

Through various methods of trapping of air-borne insects, specimens have been taken distances from land. Among the insects captured over the ocean, there seems to be a close correlation with some of the types of insects better represented on islands more distant from continental areas. Such groups include small leaf-hoppers, various types of small flies and wasps, in particular, as well as certain other small insects. Large insects or very compact ones like certain types of beetles have hardly been taken. The most southerly insects trapped were Collembola in Antarctica, and a few Araneida, Psocoptera, Homoptera, Thysanoptera, Lepidoptera and Diptera over ocean between 53° and 75° S. Lat. (38 insects trapped south of 50° and 10 south of 60° S. Lat.). In the northcentral Pacific, about 150 insects have been taken more than 400 kilometers from the nearest continent or island, at least in terms of distance from land in direction of wind source during period of trapping. About 30 cubic kilometers of air have been screened.

ÜBER INSEKTENWANDERUNGEN IM HOHEN NORDEN

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Die Literaturangaben über Insektenwanderungen in den nordischen Ländern sind recht spärlich. (Meines Erachtens sind die langen Abstände zwischen den bewohnten Plätzen eine bedeutende Ursache, denn dadurch werden viele Wanderungen gar nicht entdeckt. Man findet auch geringe Angaben über massenhaftes Auftreten von Schmetterlingen im hohen Norden.) So erwähnt z. B. Rostrup (1928), Dänemark, daß ein so dichter Schwarm von Kohlweißlingen (*Pieris brassicae*) aus einem Walde kam, daß man den Eindruck hatte, man stehe in einem Schneesturm. Köppen (1880) berichtet über Insektenwanderungen in den baltischen Staaten und auf dem Finnischen Meerbusen im Jahre 1852, wo große Mengen von Kohlweißlingen gesehen wurden. — Wenn Insekten auf Gebieten fliegen, in denen sie normalerweise nicht vorkommen, z. B. über den Gewässern, die Skandinavien und Finnland im Süden von dem europäischen Kontinent scheiden, kann ein Beobachter recht sicher sein, daß es sich um wandernde Insekten handelt.

Williams (1930) hat gezeigt, daß der Hauptteil der Wanderungen in Europa hauptsächlich in nördlicher oder südlicher Richtung verläuft. Nur wenige Insekten wandern nach Westen oder Osten. Gewässer, wo Wanderungen gesehen werden können, sind z. B. der Finnische Meerbusen, der Öresund und der Große Belt. Ornithologen haben manchmal außer Vögeln auch wandernde Insekten gesehen.

Es ist verständlich, daß sehr ausgedehnte Wanderungen nur schwer zu sehen sind, weil nur wenige Individuen zur gleichen Zeit wahrgenommen werden können. Wenn die Insekten Waldgebiete überwandern, kann ein Beobachter glücklich sein, eine Wanderung zu sehen. Darum wird die Mehrzahl der Wanderungen im Norden an den Küsten oder auf dem offenen Meere beobachtet. Von den inneren, bewaldeten Teilen von Schweden und Finnland gibt es nur sehr spärliche Angaben.

Eine der bekanntesten wandernden Falterarten in den nordischen Ländern ist die Gammaeule (*Phytometra gamma*). Sie trat im Jahre 1922 in den östlichen Teilen von Fennoskandien als Invasion auf, während die westlichen Teile nur schwach berührt

wurden. Als der Wanderinstinkt der Gammaeulen aufhörte, begann ein intensives Legen von Eiern, und dadurch wurden nach einiger Zeit viele Kulturpflanzen zerstört. Ein Viertel der Kreuzblütler gaben keinen Ertrag. Die folgende starke Einwanderung geschah im Jahre 1946, und dabei konnte man in dem südlichen Teil von Finnland auf Äckern, Wiesen und anderen nicht bewaldeten Gebieten sogar 200.000 Gammaeulen pro Hektar finden. Große Mengen von Eiern wurden in Getreideäckern auf dem Unkraut *Sochus arvensis* gelegt, welches gefressen wurde. Danach konnte man Larven sehen, die in breiter Front über die Wege auf naheliegende Äcker wanderten. Die neue Imagogeneration wurde sehr individuenreich, aber die Tiere waren beinahe hundertprozentig steril.

Die Wanderungen der Gammaeule in den Jahren 1922 und 1946 waren nach Norden oder NW gerichtet. Es konnte keine Rückwanderung festgestellt werden. Im Jahre 1949 konnte ich Ende August ein Massenauftreten von Gammaeulen auf blühenden *Solidago virga aurea* auf den äußersten Inseln des Finnischen Meerbusens sehen, und zwar auf Plätzen, wo Gammaeulen keine guten Lebensbedingungen finden, und wo ich in demselben Sommer keine Raupen gefunden hatte. Nach wenigen Tagen waren die vielen Tausende Eulen verschwunden, und ich nahm an, daß sie nach Estland gewandert waren.

Der Distelfalter (*Pyrameis cardui*), der während vieler Jahre gar nicht in den südlichen Teilen von Finnland gesehen werden kann, kommt bisweilen in ungeheuren Mengen vor. Speziell im Jahre 1948 wurden Scharen von Distelfaltern in Finnland und Nordschweden gesehen, nicht aber in Mittel- oder Südschweden. Die Wanderungen der Distelfalter wurden im Jahre 1948 in Südfinnland von mehreren Forschern beobachtet. Einige der Tiere setzten ihre Wanderung nach dem Norden und dem Eismeer fort, wo die Falter ins Wasser stürzen, wenn die Temperatur zu niedrig wird.

Während des späten Sommers können verschiedene Nymphaliden, z. B. *Vanessa antiopa*, *Pyrameis atalanta*, *Pyrameis cardui* und *Polygonia c-album* in großer Zahl an der Südküste Finnlands gesehen werden, wo sie nur spärlich als Raupen oder Imagines vorkommen. Dann verschwinden sie plötzlich, ohne daß Rückwanderungen nach dem Süden notiert werden können.

Die speziell wandernde Art, deren Wanderungen in den Zeitungen erwähnt werden, ist der große Kohlweißling (*Pieris brassicae*), der in recht großer Entfernung sicher bestimmt werden kann. Diese Art ist in einigen Jahren sehr häufig im hohen Norden, fehlt dazwischen aber vollständig über großen Gebieten. Es scheint, daß der große Kohlweißling, um eine hohe Frequenz halten zu können, eines bedeutenden Zuschusses von wandernden Individuen aus dem Süden bedarf. Dagegen wandern in Jahren mit optimaler Frequenz die Falter von Finnland nach dem Süden (Nordman, 1942).

Mehrere Verfasser behaupten, daß sich der große Kohlweißling zum größten Teil auf den baltischen Inseln vermehrt. An vielen Orten entlang den Küsten gibt es bedeutende Bestände von *Cakile maritima* und anderen Kreuzblütlern, sogar kultivierte Kreuzblütler, an die wandernde Kohlweißlinge große Mengen von Eiern legen können. Dort sind die Parasiten des Kohlweißlings sehr selten oder fehlen ganz, da sie den wegwandernden Kohlweißlingen nicht folgen können. Oft gibt es auf den äußeren Inseln des Schärenhofes keine Parasiten, die sich an den Kohlweißlingen vermehren. Auf diese Weise gelingt die Entwicklung der Raupen zu Faltern beinahe hundertprozentig.

Kohlweißlinge fliegen nur im Sonnenschein oder wenn die Sonne nur von Cirruswolken bedeckt ist. Kommen sie in den Schatten unter den Wolken, setzen sie die Wanderung nicht fort. An den Küsten des Finnischen Meerbusens gibt es sowohl im Norden als im Süden, wenn keine Tiefdrucke in der Nähe sind, Cumuluswolken.

Aber im Schärenhof, wo das Wasser die steigenden Luftsäulen abkühlt, verschwinden die Cumuluswolken wieder. Unter diesen Witterungsverhältnissen haben die Falter gute Möglichkeiten, überall, wo der Himmel klar ist, zu wandern, und können geeignete Pflanzenbestände finden, an denen sie Eier legen. In dieser Weise werden oft die Inseln des Schärenhofes zu Massenvermehrungsgebieten der Kohlweißlinge, weil sie wegen der Wolken nicht weiter ins Binnenland dringen können.

In Südfinnland hat der große Kohlweißling jährlich nur eine vollständige Generation. Die Raupen der zweiten Generation sterben, bevor sie sich zu Puppen entwickeln können. Aber in Estland ist die zweite Generation oft vollständig, weil die Kontinentalität der Witterung sehr ausgeprägt ist. Zwischen den Zonen, wo der Kohlweißling eine oder zwei vollständige Generationen hat, gibt es eine Zone, in der die zweite Generation entweder umkommt, wenn der Sommer kalt ist, oder sich entwickeln kann, wenn es warm genug ist. In den Zonen, wo sich entweder zwei oder drei Generationen entwickeln können, also nicht in den Zwischenzonen, den „Katastrophenzonen", ist die Witterung von geringer Bedeutung, dagegen können die Parasiten überhandnehmen.

Bei Untersuchungen über Insektenwanderungen fördern diese Zwischenzonen die Arbeit, denn wenn eine Generation in einem Gebiet vernichtet wird, kann durch Wanderungen zugeführtes Insektenmaterial beobachtet werden.

ON THE NATURAL LAWS OF A. MACULIPENNIS DISTRIBUTION IN VILLAGES DEPENDING ON GROUND RELIEF

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To escure its vital necessities every animal is confined to a certain natural habitat and the animal's migrations within this area may be due to changes in the environmental conditions or due to its physiological state.

Many of those animals, who migrate by air, perhaps fully use air currents to this end.

Alongside with the animals whose life is closely associated with the utilization of air streams of vast stretch (Locusta, birds, and others) there are some animals whose life cycles are also closely connected with the use of air circulations but of considerably smaller capacity and length. Their migrations are limited, as a rule, to hundreds of metres or several kilometres. As an example of such animals who naturally migrate during their life may be the malarial mosquitoes.

Two types of migrations are peculiar to the gonoactive females of the majority of *Anopheles* species, namely: from the breeding places in search of victim and to the breeding places for oviposition. In the present paper we shall dwell upon the migrations associated with searching for victims.

Two main types of hunting are peculiar to the *Anopheles* species encountered in the Soviet Union, namely: the hunting after small dispersed victims and the hunting after their large collections (V. N. Beklemishev, 1941, 1949); an example of the second type of hunting is *A. maculipennis*.

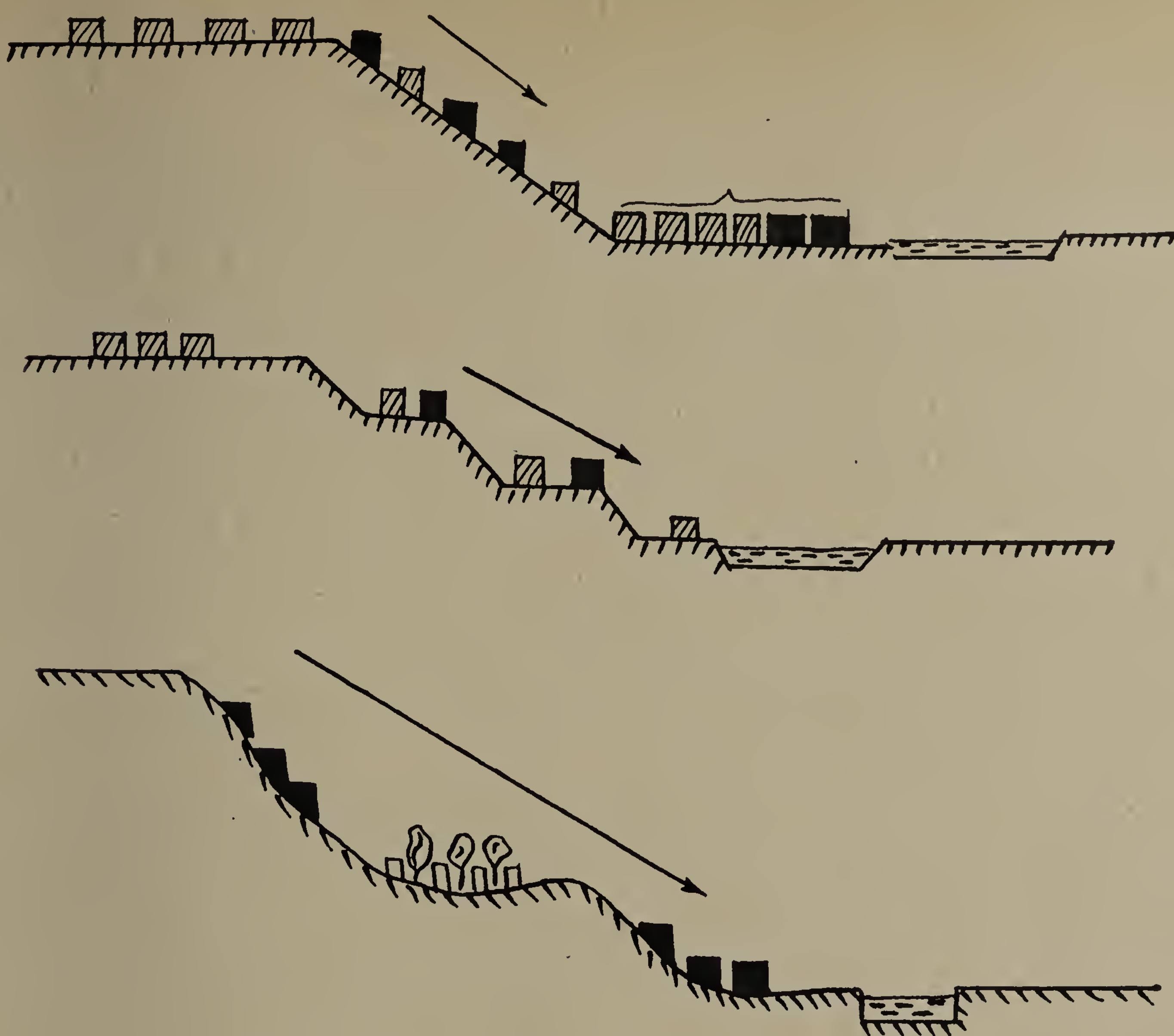

Fig. 1. Distribution of mosquitoes on different slopes.
(a) On moderately steep slope with broad above-the-flood-land-terrace.
(b) On moderately steep slope with narrow above-the-flood-land-terrace.
(c) On moderately steep slope with flattened middle part.
Black squares—homesteads with numerous mosquitoes.
White squares—homesteads with small number of mosquitoes.
Arrows—direction of air current.
Braces—no wind.
Dotted Line—river.

Since the smell is spread by air currents the peculiarities of air flow circulations, conditioned by the ground relief, may essentially influence the *Anopheles* distribution both between separate "centres of gravity" (villages) and inside them.

Most of *Anopheles* species, hunters after large victims, are endophyls with sharply expressed activities at twilight. (Distribution of other *Anopheles* species, hunters after dispersed victims usually of exophyl species, apparently, follows other natural laws not discussed in the present paper.) Therefore of special interest is the study of evening air currents when considering the natural laws of the mosquito distribution.

When studying the distribution of mosquitoes in villages depending on the ground relief, we used to let the coloured mosquitoes out with a subsequent catching them back, used to trap mosquitoes in cattle-sheds (day-time habitations of mosquitoes), and to study the evening air currents.

The peculiarities of the circulations of evening air currents stipulate the nature of *Anopheles* distribution in villages.

The absence of local air currents of constant direction and the presence of day-time strong winds usually slackening by the night to a calm are characteristic of a plain with smooth relief.

The presence of natural collections of water at a plain location of villages results in the origination of local air circulations due to different heat capacity both of water and land. Because of the smoothness of relief these currents will be of smaller length and capacity and, most probably, may influence the mosquitoes' invasion of a village in those cases when the breeding place (a natural water collection) is near the village. Besides, the micro-thermodynamic circulations will take place on any ground relief stretching between the houses and the breeding places, since the first relative to the second are always located at some eminence. These currents, however, may have an influence on the mosquitoes' invasion of the homesteads (country-houses with out-door buildings) situated near the water collections. Since the cold air drainage takes place from any convex forms, it will also stream down from any house. These micro-thermodynamic currents have an influence only on the distribution of mosquitoes within the village by facilitating their invasion of the houses. But the cold air drainage takes place only from well-streamlined protuberant forms if air currents do not pass through them. Therefore the cold air will not flow down from the sleeping-towers (pile-dwellings for sleeping at night) wide-spread in the south. The lack of thermodynamic circulations from the towers results in the fact that it becomes difficult for the mosquitoes to catch the smell of "victims" who are on the towers and as a consequence they weakly attack people sitting on the towers.

In connection with the fact that local winds of constant direction are absent on smooth relief, the smell of victim collections may diffuse in different directions: during the wind—depending on the wind direction and during the calm—evenly around the village. In consequence of this fact, on the plain, of especially great importance will be the capacity of the centre of gravity (village). Main factors that determine the character of mosquito distribution on smooth ground are the following: the distance between the centres of gravity, the capacity of separate centres of gravity and their attractiveness, the location of the centres of gravity relative to the breeding places, and the weather conditions.

At a plain location of the village the number of mosquitoes will sharply decrease when the distance between their day-time habitations (cattle-sheds) and the breeding places increases. Especially large is the number of mosquitoes at their day-time habitations near water collections, the inlets (door, window, etc.) of which are facing the breeding places.

A typical plain distribution of mosquitoes in the village may be also encountered on broken ground if the village is situated in the meso-relief area with a small inclination (on river terraces, on the bottom and gentle slopes of flat-bottomed ravines, on the plateau of the main bank, on the flattened summits of the mountains). The mosquitoes' invasion of such villages takes place under the influence of local air circulations peculiar to broken ground.

Considerable varieties in the distribution of mosquitoes as compared to those described have been encountered on the plain covered with thick forest and having damp climate. Under such conditions there has been observed the settling of a considerable part of a natural population of endophyl *A. maculipennis* mosquitoes and their dispersed distribution in the villages.

The valley dimensions, degree of its development, and profile of slopes have a considerable influence upon the location of villages.

In well-developed valleys with broad river terraces the villages may be situated on any morphological part of the valley. Usually each given village occupies only one of them, i.e. above-the-flood-land-terrace, slope or the main bank.

On the contrary, in smaller, poorly developed valleys, the villages (on the vertical) usually occupy several elements of the valley.

The location of a village, profile of the valley, and partially the character of the village (crowdness of houses, vegetation, direction of roads, and others) determine the circulation of local air currents in the village.

Observations have shown considerable differences in the mosquitoes' distribution in the villages, dependent on their location and the valley profile, conditioned by the peculiarities of the circulation of air flows.

In the mountains and on the mature erosion plain thermodynamic circulations run along valleys, gorges, ravines and across them—from the slopes.

Irregularity of the mosquito distribution by the slopes is characteristic of these landscapes. Intensive invasion of mosquitoes is observed in homesteads open to air currents; utilizing the winds of the slopes the mosquito females fly to the upper parts of the villages.

A typical plain distribution of mosquitoes is observed in the villages situated on a moderately steep slope with a broad above-the-flood-land-terrace in the lower part of the village in view of the smoothness of relief and absence of local air currents.

During vigorous breeding a considerable part of mosquitoes reaches the foot of a slope and then their further distribution in the village is conditioned by the peculiarities of the circulations of air flows characteristic of the given profile of the slope. In the villages located on a moderately steep slope with a narrow above-the-flood-land-terrace the winds of the slopes are marked at the lower homesteads and the mosquito distribution in the village depends on the peculiarities of their circulations.

In the villages situated on a moderately steep slope with a flattened middle part covered with vegetation and densely populated the numerous day-time mosquito habitations are encountered in the upper and lower houses. On the flattened middle part of the slope, in some places, the winds are absent and the mosquito females fly over it into the houses situated on the upper part of the slope.

Such profiles of the slope (for example, moderately steep slope with a narrow above-the-flood-land-terrace and moderately steep slope with a flattened middle part may be characteristic of a paradoxical distribution of mosquitoes, i.e. more numerous day-time mosquito habitations in the upper parts of the slope are at a larger distance from the breeding places (water collections).

In connection with the peculiarities of the circulation of air currents on the slopes the number of mosquitoes at their day-time habitations may not depend on the location of the inlet relative to the breeding places.

Towards the valley breezes which bring the village smells fly the mosquitoes 5 kilometres a night up the steep mountain valleys. However, at a distance of 2.5—4 km. from the breeding places the formation of local whirls ("air locks") prevents the mosquitoes from invading the villages, situated in dry tortuous valleys with developed normal profile and characteristic of them gentle incline in the lower part of the valley.

Observations have shown that the mosquitoes overcome saddles and watersheds only if there are victims in their upper parts.

Air currents in ravines with a steep bottom profile prevent the mosquitoes from flying over the ravine. The distribution of mosquitoes in the villages, cut up by such ravines, is determined by the location of the breeding places relative to the mouth of ravine.

Natural laws of the mosquito distribution in villages, stipulated by the ground relief, are confirmed by the data on the distribution of malarial patients in the villages of various types.

LONG-RANGE DISPLACEMENT OF HOMOPTERA IN THE CENTRAL UNITED STATES

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Considerable information has been published on the long-range displacement of insects such as butterflies and grasshoppers, but relatively little has been written about the long-range displacement of Homoptera. However, aphids are better known than most insects in regard to movements over short distances, as they are generally of economic importance, have complex life cycles, and depend upon wind currents for dispersal. Kennedy and Stroyan (1959) reviewed research that has given valuable information on the local flight behaviour of aphids. It is the objective of this paper to present information on the long-range displacement of both aphids and leafhoppers that occurs regularly in the central United States, and to suggest how the dispersal phenomena are dependent upon the ecology of the various species in combination with a pattern of mid-continental air movements.

Evidence of Regional Dispersals

The six-spotted leafhopper, *Macrosteles fascifrons* (Stål), is economically important on a wide range of host plants, including carrots, lettuce, celery, spinach, and flax, because of the aster yellows virus that it transmits. According to Chapman (1949) the insect may overwinter in the egg stage in Wisconsin. However, the first forms to appear in the spring are adults. Drake (1952) showed that the large spring and early summer population was due to a dissemination of adults into the state rather than to any overwintering stage. Furthermore, this population contained viruliferous individuals believed to be the principal primary source for early virus infection of susceptible crops.

Chiykowski (1958) conducted spring surveys from 1953 to 1958 in the central United States to locate the source of migrants. A large population of nymphs was found in winter grain fields in northwestern Louisiana, northeastern Texas, western Arkansas, eastern Oklahoma, southwestern Missouri and eastern Kansas. Observations in these areas indicated that heading of the grain stimulated the adult leafhoppers to leave the fields. Chiykowski (loc.cit.) found that the dispersals were closely correlated with the airflow patterns that prevailed during periods when flights occurred. In some years the leafhoppers apparently moved up to 1,000 miles.

The potato leafhopper, *Empoasca fabae* (Harris), is a serious pest of alfalfa, beans, potatoes and other host plants. On alfalfa, the species produces a toxicogenic disease known as "alfalfa yellows". Heavy infestations reduce the quality and quantity of hay. The leafhopper does not overwinter in the North Central Region, but each spring the population is reestablished by migrants. Medler (1957) reported on the cooperative research that first determined the major pattern of seasonal dispersal.

The potato leafhopper overwinters in the Mississippi delta area. A population increase occurs in this area during February, March and April. As the legume host plants are cut, plowed under, or become dry and tough in early April the adults are launched on days when temperatures are suitable for flight. The air-borne leafhoppers are then carried northward by prevailing currents of warm wind. This northward movement has been determined in the central United States since 1951 (North Central Regional Project NC-29). Entomologists in the various states have cooperatively investigated the flight activity each spring by means of field sweepings, aerial collecting devices, and inspection of favored host plants, such as *Caragana* sp. and seedlings of walnut, *Juglans* sp. Glick (1960) studied the movement by airplane trapping, and found specimens at altitudes from 200 to 4,000 feet. The majority of the specimens were collected during the morning flights of the airplane.

Several species of aphids are economically important on cereal crops. In addition, they may transmit the yellow dwarf virus disease that seriously injures barley and oats. The very destructive greenbug, *Toxoptera graminum* (Rondani), the English grain aphid, *Macrosiphum granarium* (Kirby), the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), and the apple grain aphid, *Rhopalosiphum fitchii* (Sanderson), are common in the North Central Region. The initial build-up of these species in northern states is the result of a spring dispersal of alates from overwintering areas in states located farther south. Glick (1960) listed many aphids in airplane collections, including the English grain aphid and the apple grain aphid. Although the apple grain aphid can overwinter in Wisconsin on its winter host, Orlob (1959) found that alate aphids occurred on summer hosts at a time when fundatrices were just hatching on the winter host. These alate individuals in the grain must have been dispersed from a southern area where the aphids had sufficient time for an earlier population build-up. The corn leaf aphids which are dispersed into Wisconsin establish themselves mostly on barley. Later in the season a widespread dispersal to corn plants takes place locally when the corn has matured sufficiently to support the species.

Orlob and Medler (1960) reported that the wind directions from southern areas were favorable at the time grain aphids first appeared in Wisconsin. Low pressure centers located over southwestern Kansas caused winds that rotated over northern Texas, Oklahoma, Arkansas and southern Missouri, into Wisconsin.

Medler and Smith (1960) reported on an outbreak of the greenbug in Wisconsin that was associated with a heavy dispersal of air-borne aphids from the south. Data obtained from yellow pan traps in Missouri, Kansas and Wisconsin demonstrated that the dispersal activity included a large area of the midwest.

At the present time research is being conducted on the dispersion of aphids in the central United States. The flights are being studied by means of the yellow-pan water traps first developed by Moericke (1951). About 100 pans are located in eleven states. The data obtained with the yellow pans is expected to provide information on, (1) the regional dispersion pattern, (2) the relative attractiveness of yellow pans to different species, (3) relation between aphid abundance, meterological conditions and population build-ups.

Regional Distribution and Abundance of Host Plants

Abundant acreages of cultivated host plants are provided for the six-spotted leafhopper and various grain aphids in the central United States, as this is the principal region of small grains in the country (Figure 1 A, B, C). A hard red winter-wheat region centers in Nebraska, Kansas, Oklahoma and the Texas Panhandle.

The hard red spring-wheat region includes North and South Dakota, western Minnesota, and adjacent parts of Canada. Oats, which rank second in importance to wheat, are produced in a broad region that circles south of the Great Lakes. As oats are generally used in rotation with corn, the greatest concentration coincides with the center of the Corn Belt. The barley acreage which is concentrated in the spring wheat region is usually limited because of its poor competitive relations with other feed grains.

There are vast acreages of leguminous host plants suitable for the potato leafhopper. The largest acreages of alfalfa in the United States are in the midwest (Figure 1 D), with Wisconsin, Minnesota and Nebraska leading the states, and together accounting for over 25 per cent of the nation's alfalfa acreage. Approximately 10 per cent of Wisconsin's 10 million acres of harvested crops is tame hay, which includes alfalfa, clover, or legume-grass mixtures. In most years Wisconsin leads all states in tame hay acreage and tonnage produced.

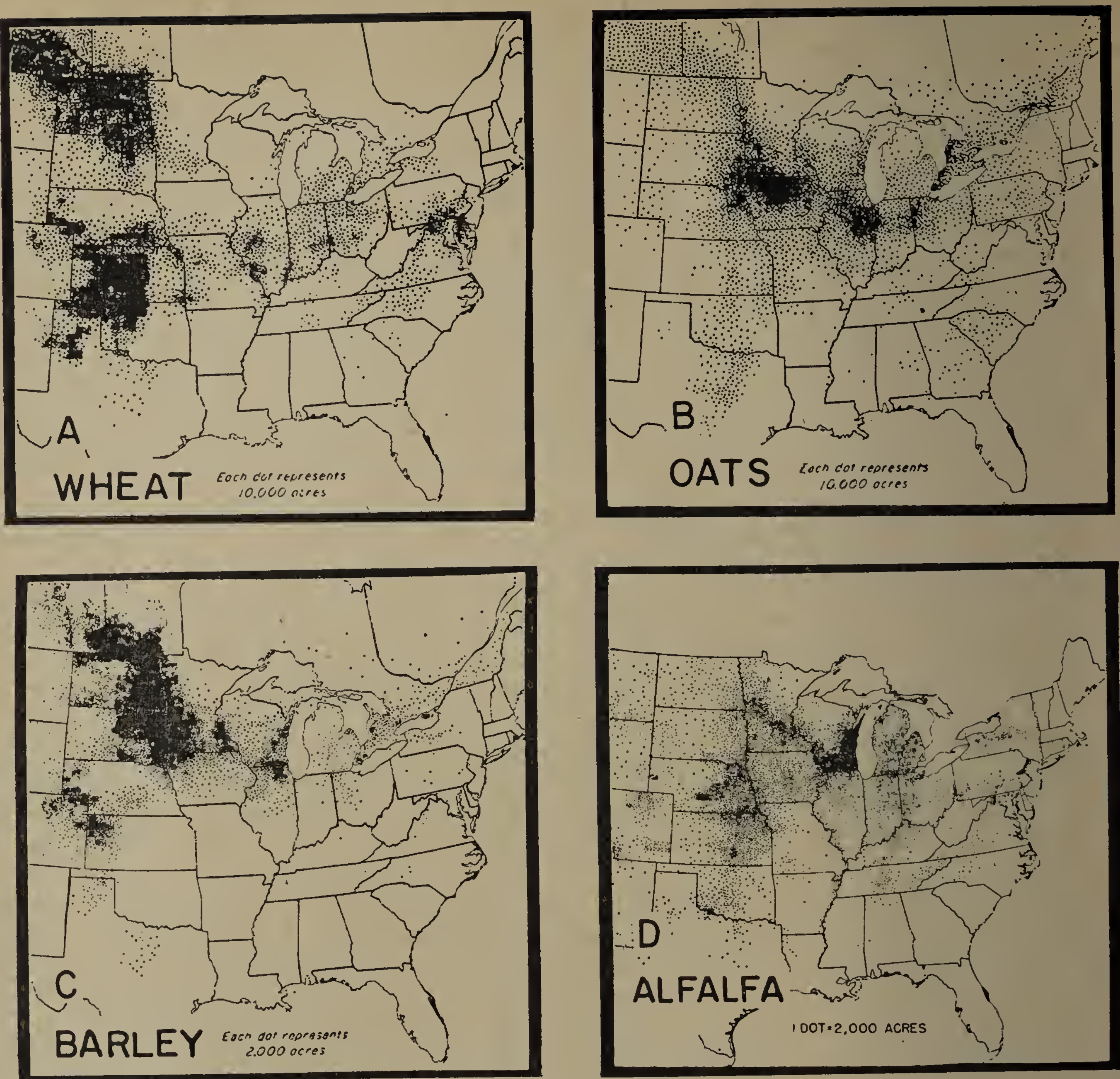

Fig. 1. The principal regions of small grain and alfalfa in the central United States.

Winter host plants in the southern region that support large populations of leafhoppers or aphids become unsuitable as they mature or are harvested progressively from south to north. This may be the stimulus for dispersal. In northern states, host plants in a suitable condition for the support of insects are lacking during the winter. However, with the advent of spring many thousands of acres of small grains are planted. The young grain provides a highly attractive feeding environment for the six-spotted leafhopper and the aphids. The spring growth of legumes provides an abundance of succulent vegetation also highly favored by the potato leafhopper. Therefore, the establishment of the migrant population is generally successful because of the widespread availability of food. However, the subsequent development of populations in any local area would be influenced by many factors in addition to the plant host, such as the density and condition of the first migrants, reinforcement by later migrants, and weather conditions.

Source Areas of Migrants

Figure 2 A shows that winters in the hard red winter-wheat region are moderately cold. Winter populations of the grain aphids and the six-spotted leafhopper are not eliminated in this region especially along the southern border. The topography in the

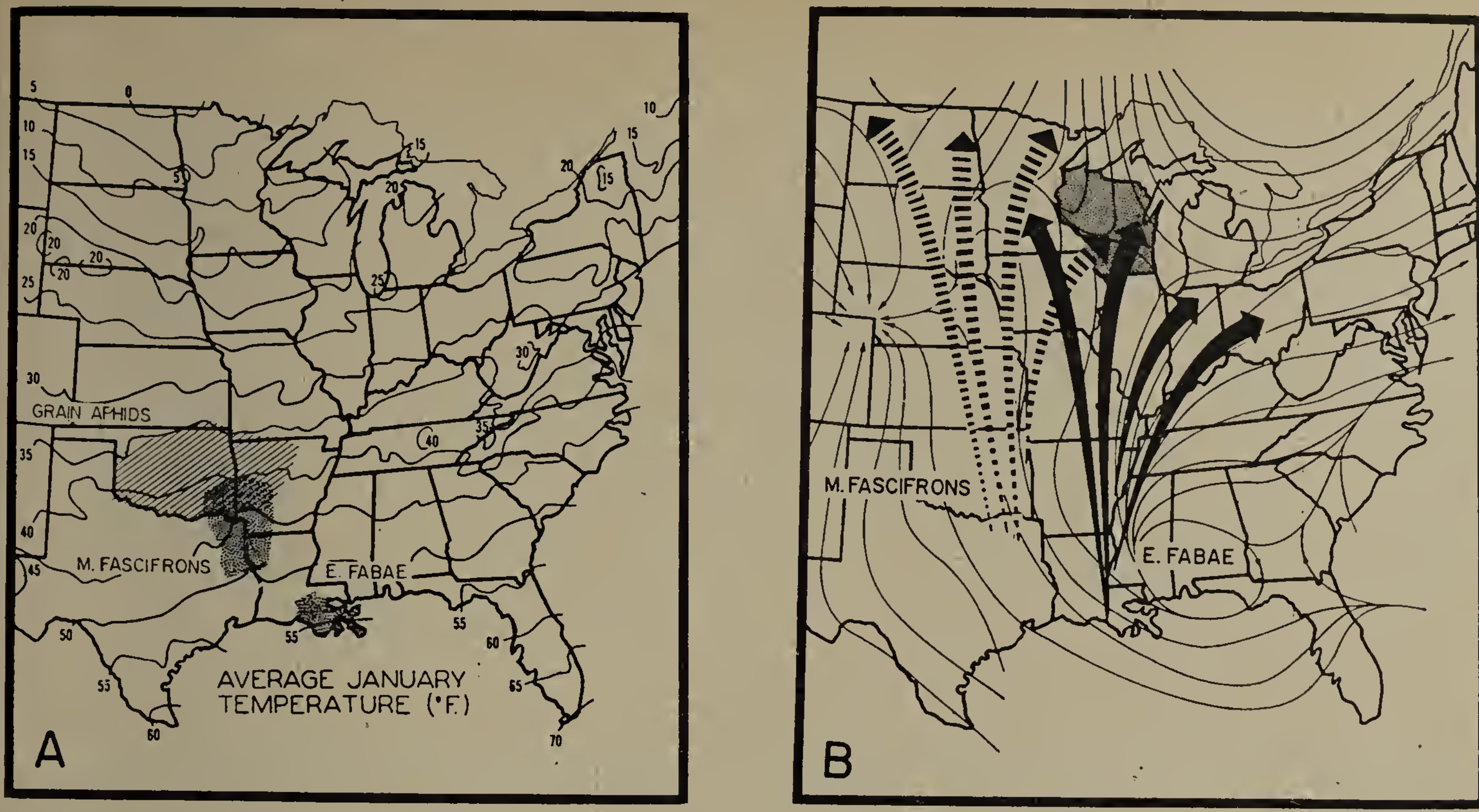

Fig. 2A. The January isotherms in the central United States and the major sources of the grain aphids and leafhoppers that are dispersed northward each spring.

Fig. 2B. The spring dispersal paths of *Macrosteles fascifrons* (Stål), the six-spotted leafhopper, and *Empoasca fabae* (Harris), the potato leafhopper.

southeastern part of this region is hilly. Numerous valleys exist which offer sheltered environments for the early growth of grain and the early development of the insects.

Painter et al. (1954) stated that the greenbug is known to have passed some mild winters as far north as Manhattan, Kansas. Dahms (personal communication) found all of the above-mentioned species of grain aphids during December and January in southwestern Oklahoma. In most years the 35° F. isotherm would seem to be associated with the northern limits of the overwintering populations of the grain aphids. The area with a winter breeding population of adult six-spotted leafhoppers is not known, but numerous eggs occur in favored host plants south of the 40° F. isotherm. Eggs also may be found as far north as Wisconsin. The 50° F. isotherm probably is coincident with the greatest northward extension of the overwintering area of adult potato leafhoppers. The various wintering areas that are indicated in Figure 2 A are, in most years, the principal source of the first migrants in the late winter or early spring.

Air Currents of the Central United States

The air currents existing at the time of a dispersal must be studied to determine the source and route of air-borne insects. However, a general understanding of the spring dispersal can be gained by an analysis of the normal air currents which dominate the central United States during March to June. Diagrams that are given in Figure 3 are based on data of surface resultant winds taken at anemometer height (Lahey and Bryson, unpublished). The streamlines are applicable up to 1000 feet above ground level.

The diagrams show clearly that the northward flow of warm southern air is blocked by polar Canadian air masses. In March (Figure 3 A) the warm southern air is turned eastward before much penetration occurs to the north. In April (Figure 3 B) the northward flow of warm southern air is stopped by the polar Canadian air along a line that runs through southern Nebraska, central Iowa and northern Illinois. The warm southern air that is funneled to the north is divided so that currents are turned east and west of a line formed by a narrow wedge of cold air. In May (Figure 3 C) the funnel of

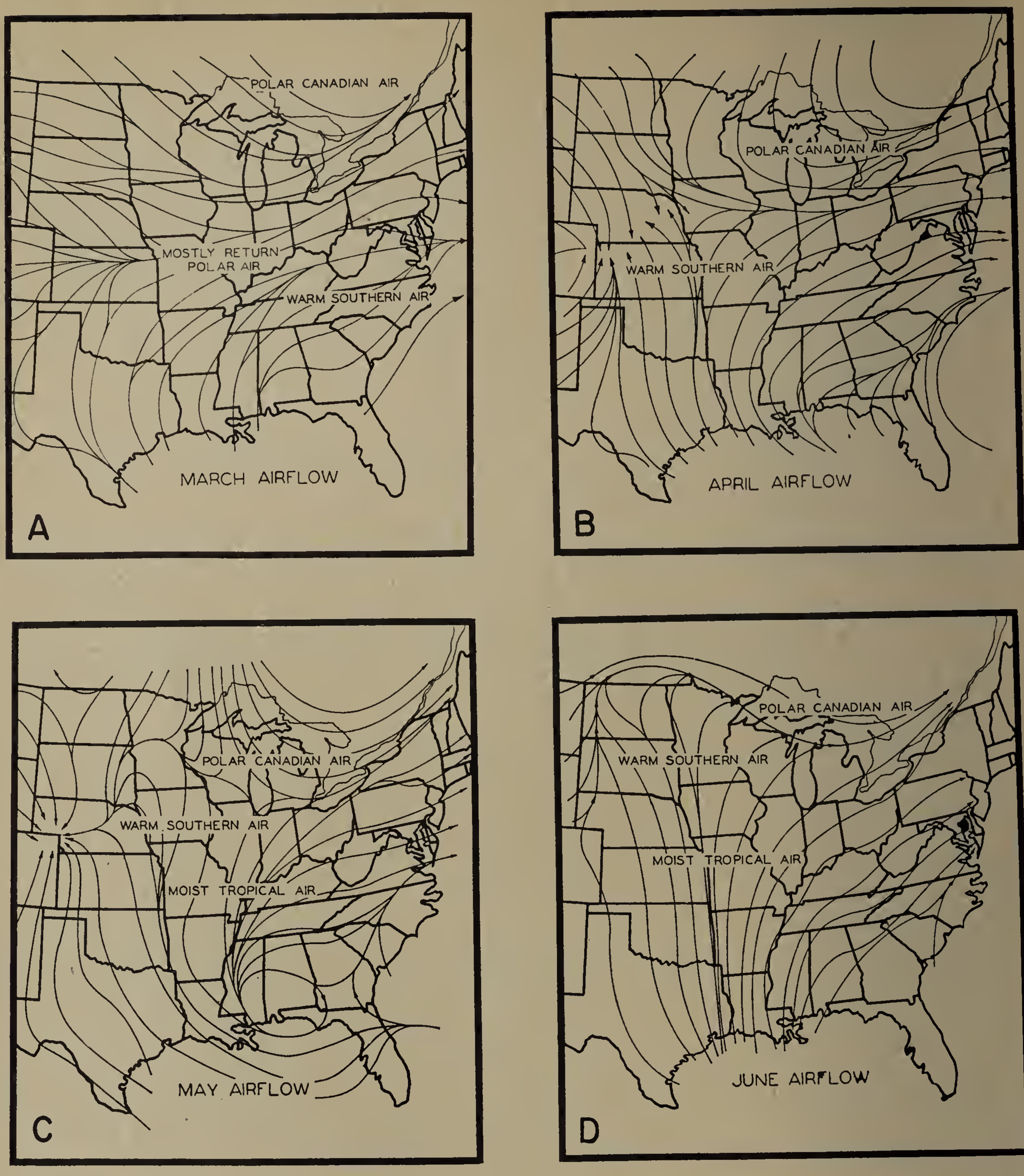

Fig. 3. The average surface resultant winds in the central United States during March to June.

warm southern air has widened considerably and it forms a blunt wedge in the cold air mass. It can be readily seen that the warm southern air flows in a broad band from the Gulf of Mexico as far northward as central Minnesota and Wisconsin. In June (Figure 3 D), the southern air flow covers the entire midwest and part of central Canada.

It should be emphasized that the diagrams of the air currents represent only the average flow of air for the particular month and are subject to variation from year to year. It is not uncommon that the pattern for one month may lag or be advanced for several days in another month. Also, variations occur in the location of the streamlines and the wedge of warm air.

A comparison of the airflow patterns with data on aphid and leafhopper dispersals indicates that close relationships exist. For example, in Wisconsin the first potato leafhoppers are found in the southwestern part of the state under conditions of the May

airflow. The potato leafhopper is found infrequently in Kansas and Nebraska, probably because the streamlines crossing the winter breeding areas do not flow that far westward. In most years the main dispersal of the potato leafhopper follows the Mississippi river valley in almost a direct line from Louisiana to Wisconsin (Figure 2 B).

On the other hand, the six-spotted leafhopper usually reaches Wisconsin earlier than the potato leafhopper, probably because the winter breeding areas are closer. The normal dispersal pattern of the six-spotted leafhopper (Figure 2 B) along the Arkansas—Oklahoma and Missouri—Kansas borders almost exactly coincides with the streamlines crossing the area. These air currents determine the northwesterly direction of the dispersal. However, in some years the path may swing northeasterly through Wisconsin. In May, additional source areas in Missouri and eastern Kansas produce leafhoppers that funnel out widely farther north in the Dakotas, Minnesota and the Prairie Provinces of Western Canada.

LITERATURE

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A FLIGHT OF INSECTS IN THE GULF OF ADEN

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These observations were made three years ago, during a sea voyage from Singapore to the eastern seaboard of Canada. The journey was made by freighter, S. S. "Ampenan" (Royal Rotterdam Lloyd Ltd.), and I take this opportunity of thanking the Captain and officers for their kindness in supplying meteorological and positional data. Thanks are also due to the Director of the Commonwealth Institute of Entomology and his staff, who made this paper possible by identifying all the insects mentioned.

Material

At 9 a.m. on June 10, 1957, insects began to appear about the ship while westward bound for Djibouti and deep within the Gulf of Aden. The position was 11° 52′ N, 44° 27′ E, the nearest land (Aden Protectorate) being 87 km. to the north. A light breeze was blowing from the NE, i.e. from the direction of Arabia, and northerlies had

prevailed throughout our 24 hours in the Gulf. The closest part of the African coast (Fr. Somaliland) was 122 km away. Numerous moths, beetles and hemipterans landed aboard during the next hour, in which 30 km were covered. In addition, several dragonflies flew close to the ship in this period, but none alighted. The following species were identified.

	Hemiptera
Lygaeidae	*Aphanus apicalis* Dall.
	Lepidoptera
Agrotidae	*Rhesala moestalis* Walk.
	Laphygma exempta Walk.
Geometridae	*Traminda neptunaria* Guen.
	Coleoptera
Carabidae	*Calosoma senegalense* Dej.

A lygaeid (*Dieuches* sp.), the identity of which could not be established at the British Museum, an undetermined geometrid and a second carabid were also collected; while a leafhopper (Hemiptera/Homoptera) was noticed but not secured.

Discussion

At least ten species of insects of four orders were thus included in the mixed flight, the Lepidoptera predominating. Several moths could be seen in any direction, from just above the water to funnel height, throughout the hour of the incident, on conclusion of which some 300 (mostly *Laphygma exempta*) were scattered over the decks. The moth landings were notable for two things, initial hyperactivity that rendered it most difficult to capture specimens by hand, and the fact that within minutes of settling down the insects were collapsing on surfaces no more than warm to the touch.

Such a picture of stress was not presented by the Hemiptera and beetles. The latter ran about actively, took intermittent short flights, and far from dying of exhaustion, a *Calosoma senegalense* placed in a screw-capped vial scarcely larger than itself actually proved to be still alive on arrival in Montreal 18 days later!

Calosoma, a genus of largely arboreal carabids, includes a species (*C. sycophanta*) introduced into the U.S.A. from Europe for gypsy moth control (Burgess, 1911). A tree-climbing habit might have led to the involuntary participation of such unlikely insects as these aberrant ground beetles in the flight, together with leafhoppers and other forms presumably snatched from vegetation by a gust of wind.

Economically, more interest attaches to two of the other members of this group encountered almost midway between Arabia and Somalia. Another species of the lygaeid genus *Aphanus*, *A. sordidus* F., is a pest of shelled ground nuts in Nigeria (Evans, 1952), and the dominant moth, *Laphygma exempta*, is of major importance. Evans (1952) lists it as one of the greatest pests of pastures, forage grasses and cereals in Africa, and refers to a South African suggestion that the insect is not permanently established in the Union but periodically migrates there from outside. While the present observations are evidence for this moth's susceptibility to mass airborne dispersal, there was no sign of any directional impulse. The initial hyperactivity succeeded by complete collapse suggested strain to the limit of the insect's resources in accord with sudden removal (quite possibly some considerable time previously) from the habitat. These facts, and the mixed nature of the flight, combine to indicate involuntary dispersal through the agency of winds.

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CARACTERES PARTICULIERS DES MIGRATIONS DU HANNETON COMMUN (Melolontha melolontha L.)

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Les migrations du Hanneton commun (*Melolontha melolontha* L.) présentent un caractère stéréotypé. Nous avons déjà montré l' «imprégnation» indélébile de la direction choisie au cours du vol préalimentaire, celle-ci se maintient au moment de la migration de ponte, mais en changeant de sens, elle reprend son état primitif après le dépôt des œufs, toujours indépendamment de tout repère visuel terrestre (1).

De nouvelles expériences apportent des preuves complémentaires et permettent d'éliminer certains facteurs que l'on pouvait considérer comme servant de guide à l'insecte.

1° Des élevages en conditions artificielles ne modifient pas la direction du vol de ponte.

a) En 1959, des femelles, provenant d'un vol préalimentaire crépusculaire dirigé vers l'est, ont été élevées dans une cabine recevant un éclairement artificiel diffus dont l'intensité est constamment au voisinage de 100 lux. 10 jours $^1/_2$ plus tard, alors qu'elles sont pleines d'œufs, elles sont lâchées le matin dans un site inconnu. Huit sur dix prennent leur envol vers l'ouest et le nord-ouest, c'est-à-dire dans le sens inverse de leur première sortie de terre.

b) En 1960, des femelles, récoltées dans la région de Dijon le soir au cours d'un vol préalimentaire en direction E., ont été élevées à Colmar dans une cabine recevant une luminosité très faible et maintenue constamment au voisinage de 0,5 lux. Au bout de 13 jours, lorsqu'elles sont prêtes à pondre, elles sont libérées à Colmar (c'est-à-dire à 180 km de distance, soit à 0°75 de latitude N. et à 2° de longitude E. par rapport au site d'origine). 13 bêtes ont été suivies, 10 partent vers l'W et le SW comme les témoins élevées en lumière naturelle.

Dans ces expériences, les insectes en élevage étaient à l'abri de toute radiation lumineuse naturelle et ils connaissaient à peine l'astre du jour car celui-ci a déjà disparu au-dessous de l'horizon au moment du vol préalimentaire. Fort peu, en effet, restent en surface une journée entière avant de prendre leur départ, leur attente se réduit généralement à quelques heures en fin d'après-midi.

2° Il a été procédé à des dépaysements de grande ampleur dans l'espace ou dans le temps pour lesquels les modifications intervenues dans le mouvement apparent du soleil, quoique sensibles, sont cependant encore trop faibles pour que leur influence sur la direction prise par les Hannetons puisse être perçue par l'observateur.

a) en mai 1959 une centaine de femelles prises le soir en migration de ponte à Rouffach (Haut-Rhin) ont été lâchées, par M. Rambier, 4 et 5 jours plus tard le matin à Montpellier (Hérault) à 550 kms plus au sud (soit à 4°' 30 de latitude S. et à 3°' 30 de longitude W. par rapport au lieu de capture). Elles sont presque toutes reparties vers l'ouest selon la direction originelle.

b) la direction du vol de ponte est encore conservée (le matin dans un site inconnu situé à 14 km au nord du lieu de récolte) après un choc de froid (2 jours $^3/_4$ à — 3° ou à 0°) suivi d'un séjour à l'obscurité complète (2 jours à 15—18°).

c) des femelles pondeuses maintenues pendant 28 jours ½ dans l'obscurité, dont 27 ½ à + 2°, se retrouvent encore au cours d'un lâcher effectué dans les mêmes conditions que dans l'expérience b.

3° L'influence du magnétisme sur l'orientation a été examinée au printemps 1958, grâce à l'aimable concours de l'Institut de Physique du Globe et du Laboratoire de Physique de la Faculté des Sciences de Strasbourg. Il s'agissait de vérifier l'hypothèse

émise à ce sujet par F. Schneider (2). Des séries de bobines de 1 m ou de 0,50 m de rayon, parcourues par un courant électrique d'intensité connue, produisent un champ magnétique en vue de modifier la composante terrestre, et le cas échéant, de l'annuler ou de l'inverser.

Des Hannetons capturés dans la région de Wissembourg (Bas-Rhin), aux vols de migration du matin ou du crépuscule, ont été relâchés quelques instants après, au milieu de ces dispositifs placés en plein air. Si le ciel est visible, les insectes retrouvent leur direction et ils ne sont pas influencés par les variations du champ magnétique. Les bêtes sont désorientées dans un local complètement clos, avec éclairage artificiel, même si le magnétisme terrestre n'est pas modifié.

Des femelles capturées sous filet ont été mises pendant 20 minutes, avant le vol préalimentaire, dans l'entrefer d'un puissant électro-aimant où elles ont subi respectivement des champs intenses de 10.000 et 20.000 gauss sans paraître incommodées (le champ mangétique terrestre est beaucoup plus faible: 0,2 gauss environ). Un ou deux jours après, elles furent relâchées en plein air dans un site inconnu. Leur comportement a été semblable à celui des témoins non traités et elles ont présenté le même manège caractéristique au cours du vol d'orientation.

D'autres bêtes prises au vol de ponte ont été placées pendant 20 minutes dans un champ de 4000 gauss. Elles ont presque toutes retrouvé leur direction quelques jours plus tard.

De petites aiguilles d'acier aimanté de 5 à 6 mm de longueur produisaient chacune, à 10 mm de leur centre, un champ au moins égal à 2 gauss, c'est-à-dire plus de 10 fois supérieur au magnétisme terrestre. Ces aiguilles ont été fixées de différentes manières sur le dessus du pronotum (à proximité immédiate de la tête) de femelles pondeuses. Au moment des lâchers de dépaysement, les insectes ont repris leur ligne de vol tout comme les témoins.

Il est délicat de tirer des conclusions certaines de résultats négatifs, toutefois on peut affirmer que le Hanneton s'est montré insensible à des champs magnétiques intenses et que son comportement n'est pas modifié par des variations importantes du champ terrestre.

4° Il a été procédé en mai 1958 à des dépaysements dans l'aérodrome militaire de Meyenheim situé à 125 kms au sud du lieu de capture. Les insectes pris au vol de ponte ont été lâchés à 500 m de puissants appareils radar à impulsions émettant des ondes radio-électriques d'une fréquence de 9368 mégacycles par seconde (soit une longueur d'onde de 3,2 cm).

Les femelles ont pris leur envol sans paraître gênées, et pour la plupart, sont reparties comme les témoins libérés en dehors du faisceau du radar. Celà signifie que les insectes ne possèdent pas d'organes sensibles à ces ondes, mais ils pourraient être réceptifs pour d'autres longueurs d'ondes de l'ordre du millimètre par exemple.

La faculté d'orientation du Hanneton commun ne semble pas être en relation avec le magnétisme terrestre. Elle se maintient malgré l'absence, pendant plusieurs jours, de tout agent physique naturel actuellement connu, d'origine externe, et susceptible de fournir des indications sur le déroulement du temps (rythme de 24 heures, mouvement apparent du soleil, etc. . . .) mais elle se manifeste seulement à l'air libre. L'insecte a besoin de la vue du ciel et d'un grand espace pour retrouver sa direction en utilisant le soleil et d'autres repères cosmiques dont la nature est difficile à préciser.

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MIGRATION OF COCCINELLIDS TO THEIR HIBERNATION QUARTERS

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The aggregation-habit has long been known in Coccinellids. And besides a vast number of purely descriptive reports also some papers appeared trying to explain this feature. Although many interesting ideas have arisen, no explanation quite satisfying has been given, as mentioned also by Bodenheimer (1943) and Williams (1958).

This migration and aggregation behaviour seems to be most outstanding in two Coccinellids: *Hippodamia convergens* Guer. and *Semiadalia undecimnotata* Schneid. On the basis of four years' observation of the latter mentioned species, the hibernation quarters of which were found for the first time in Europe (and are analogous to those in Central Asia recorded by Dobzhansky [1925]), I can conceive at least in a rough outline the mechanism of this interesting feature.

In the midsummer the internal and external factors result in changing the physiological condition (Hodek, Čerkasov 1958, 1960) and the behaviour of the Coccinellids. After living solitary and being attracted to certain places only for food, the beetles do not feed any longer and move in late July and early August already to the hibernation quarters, where they are swarming, Obviously the completion of the accumulation of appropriate reserves of fat and glycogen make the migration instinct operative. In colder years when this process is retarded also the migration to the winter quarters is delayed. The beetles of both sexes move to their hibernacula gradually so that the coming takes usually two or three weeks, taking place only on warm sunny days, mostly in the afternoons.

As quarters such places are chosen as crevices in or at the bases of projecting formations, situated mostly in the top area of a hill or mountain. No hibernation quarters of *Semiadalia undecimnotata* have been found till now in the plains. It seems to be obvious that two factors participate in directing the beetles; the most outstanding is the manifestation of an instinct, similar to that of the swarming pairing ants (Chapman 1954) or other insects, i. e. to seek conspicuous isolated features in the landscape and to swarm round them. When the temperature has fallen, they stop their activity and aggregate somewhere in a crevice of this formation. But, on the other hand, perhaps also the air-currents will be of some importance, although, indeed, nothing is known as to their influence in this respect.

The fact that the same places have been selected every year has been explained through the orientation of the coming beetles to the bodies of the Coccinellids having died during the previous hibernation season. We do not accept this explanation because of having ascertained that the beetles have taken aim at an other artificially built formation if this one was more outstanding than the one previously used. It is, on the other hand, very probable that those coming later are attracted chemotactically to the former inhabitants, having arrived there several days before. Thigmotaxis cooperates with the chemotaxis in leading the beetles into aggregations and enables the hiding in crevices, cracks, fissures etc.

At present, making use of isotopes will naturally help to elucidate some unsolved problems, as e. g. the distance of migrations to the hibernation places and similar ones. It is, however, to be feared that other questions which are not very suitable for an experimental approach will remain unsolved for a long time yet.

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AN HYPOTHESIS ON THE MIGRATION OF THE SPINY COTTON BOLL WORM EARIAS INSULANA IN ISRAEL

E. RIVNAY

Manuskript nicht eingelangt

ABSTRACT

The notorious cotton pest, the spiny boll worm, *Earias insulana*, is distributed all over the African continent. It extends further north into southern Europa, and eastward into western Asia, Arabia and India. Although this species is indigenous to Israel, and it is capable to survive the winter there, nevertheless, flocks of this moth from out of the country invade Israel from time to time, and reinforce the local population. This hypothesis is based upon 1. The discrepancies in the occurrence of heavy infestation in different sections of the country, and which is variable every year. 2. Upon trapping in semi desert areas such as Eilat. The writer believes that the invasion of this species into Israel may take place in two routes. 1. From Egypt through northern Sinai—to the Coastal Plain. 2. From the western coast of the Read Sea through the Gulf of Akkaba to the Jordan Valley. These invasions being from different sources, may be independent in the time of their occurrence hence the discrepancies in the localization of the heavy infestation.

MIGRATION OF FLYING DIPTERA

B. HOCKING

Manuskript nicht eingelangt

ABSTRACT

Five other topics selected for symposia at this congress have a direct bearing on this subject. First, insect acoustics. The very limited range of wing-beat frequency found in insects of a given species and similar physiological condition and the fact that most migrations, or at least most that man observes, are carried out in close order, suggest that resonance between individuals may be an important factor in migration. Second, the chemistry of insects is relevant in that facts, the most favourable energy source for migration, are the chief fuel in most primitive and large insects, while carbohydrates apparently take this role in the Diptera. Third, the host-seeking behaviour of mosquitos and some other blood-sucking insects seems initially to involve an anemopositive response conditioned by olfactory stimuli. Medium to long-range translocations may result from this before a blood meal is obtained. Fourth, blood protozoa of wild animals transmitted by insects depend on the travel of the insect between one animal and another, or on repetitive consummation of host-seeking behaviour. The translocations which result depend on the distances between hosts, that is on population density. Fifth, insect life in large towns, recent consideration of a mosquito problem in Edmonton suggests that the host seeking behaviour described above may operate on a macro scale to draw numbers of mosquitos into a large town. Recent data on the magnitude of the migrations of Diptera and recent work on behaviour and its interactions with weather in bringing these migrations about and directing them is reviewed.

MIGRATIONSVERSUCHE MIT DROSOPHILA

H. BURLA

Manuskript und Abstract nicht eingelangt

MIGRATION AND FLIGHT RANGE OF WATER BUGS WITH SPECIAL REFERENCE TO THE CORIXIDAE

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The Hemiptera-Heteroptera originally evolved as terrestrial insects, but a number of families have subsequently invaded freshwater and brackish habitats and become adapted to a wide range of conditions. Families such as the Notonectidae and Corixidae have well developed powers of flight and are an important constituent of the fauna of temporary habitats. The migratory habits of these water bugs are, therefore, better known than those of other aquatic Hemiptera. In a number of the Nepidae and Naucoridae the flight muscles are reduced and migration is a rare phenomenon in these families. For these reasons, this paper is mainly concerned with the migration of Corixidae and Notonectidae.

Migration

In the summer months, during warm, sunny windless periods the following diurnal changes, observed in a small Lancashire pond may be taken as typical of many temporary habitats.

The light intensity mainly increases between dawn and 9.00 hours (G.M.T.) attaining its maximum value just after 10.00 hours. There is a rapid rise of water temperature between 9.00 hours and noon after which the temperature remains approximately constant until 15.00 hours. From 9.00 hours onwards a thermal gradient gradually develops from the surface. After 15.00 hours the temperature of the surface declines, while that of the bottom continues to rise. At about 16.00 hours a uniform temperature is produced, and the habitat-temperature then slowly declines until just after dawn the following day. The main decrease in the intensity of the light occurs between 17.00 hours and sunset.

Water absorbs light, especially when fine colloidal particles of soil or detritus are present in suspension. In all aquatic habitats, the light intensity varies inversely with the depth. When the habitat temperature is uniform, corixids move along the bottom, down this dorsal light-intensity gradient, into deeper water, but move in the opposite direction when a thermal gradient develops from the surface.

As the insects move in-shore and their temperature rises, the efficiency of the superficial gas stores, acting as physical gills, declines. This causes the insects to surface more frequently and to become increasingly directly dependent upon the atmosphere for their oxygen supply. Studies on the behaviour of corixids during surfacing have shown that these insects become positively phototactic, when deficient in oxygen and negatively phototactic when this oxygen deficiency is removed (Popham 1959). This decline of physical gill efficiency is associated, therefore, with an increased tendency to become positively phototactic and at a critical stage the insects settle on the surface or habitat edge to prepare for flight.

Water bugs may migrate as early as 9.00 hours (Popham 1952), but normally do so mainly between 10.30 and 13.30 hours. When weather conditions are less stable, migration may be delayed until 16.30 to 17.30 hours (Macan 1939).

Although a rising temperature is normally the main factor stimulating water-bugs to emigrate, other factors may, on occasions, be equally effective. During periods of drought, small temporary habitats decrease in size and the population becomes concentrated in increasingly smaller areas. This causes the insects to jostle one another and in this way the activity level of the population tends to increase. The insects may then be stimulated to fly by a sudden increase in the light intensity, such as may be produced by the passing of a cloud. When corixids are kept in jars for a few hours, after being collected in the field, and the bugs are later transfered to sorting trays in the laboratory, species such as *Corixa nigrolineata* Fieb. and *C. lateralis* Leach fly towards the brightest source of light. Many water bugs can also be induced to fly by placing them on dry land, when it would appear that the weight of the body on the legs stimulates the insects to fly.

As water bugs leave their former habitat, the initial flight path is towards the light (Popham 1952), but the insects will fly with the wind, if its speed exceeds about 5 km. per hour. By being positively phototactic in these early stages of migration, water bugs are able to rise clear of the vegetation and other obstacles surrounding the habitat, before the main flight path is adopted. Migrating water bugs discover and locate new habitats by the light reflected from the water surface. When reflected light is perceived by the lower ommatidia of the eyes, the insects change their flight direction and fly downwards towards the new habitat (Popham 1953). On breaking the water surface, the bugs swim to the bottom and the migratory flight is completed. If the bugs fail to find a new habitat, they continue to fly until just after dusk and then fall to the ground (Uvarov personal communication). Corixids caught in light traps at dusk are still active the following morning (Lansbury per. com.) but whether they can then migrate and could continue to do so for several days is not known.

Conditions favourable and unfavourable to migration

Aquatic Hemiptera-Heteroptera can only fly, when the following minimum air or water temperatures are exceeded. Thus small corixids, such as *C. nigrolineata* and *C. lateralis* Leach only fly at temperature of 12° C or more, the medium sized corixids, such as *C. praeusta* (Fieb.), *C. linnei* (Fieb.) and *C. sahlbergi* (Fieb.) at temperatures exceeding a range of 15° to 18° C, while the larger water bugs such as *C. punctata* (Illi.) and *Notonecta glauca* L. require a minimum temperature of about 18° C before flight is possible.

The facts given in the previous section show that corixids are most likely to migrate during periods of windless, warm, sunny weather and it is interesting to note that it is under such conditions Poisson, Richard and Richard (1958) found corixids to be most abundant in their light traps.

In small temporary habitats winds of only 5 to 8 km. per hour, cause the water to circulate keeping it oxygenated and of a uniform temperature. When the sky is cloudy, thermal gradients do not normally develop, while rain keeps the habitats full of water. Under these conditions, corixids move down the dorsal light gradient into deeper water and emigration does not normally occur.

In the temperate regions of Europe and North America, many corixids and Notonectids migrate mainly during the spring and autumn. Migration does not normally occur in the winter months, because the air and water temperatures are too low. Over wintered adults, mainly females, migrate in March and April and thus distribute the

species during the mating season. Adults of smaller corixid species, which have two or more generations per year, may migrate during the summer months, but the main period for migration occurs from July onwards, when the last brood of adults appear. At this period there are equal numbers of male and female migrants.

Flight Range

By making water bugs fly in still air over a known distance at 20—25° C towards a light source, it was found that *C. distincta* flies at 1.28 m/sec and *C. punctata* at 1.46 m/sec (Popham 1951).

The flight range of Corixidae can be estimated from the times they normally leave their habitats, their flight speeds and the times of arrival at new habitats or light traps. Richardson (1907) observed that corixids arrived in a pond between 11.00 and 14.30 hours, while Lange (1905) observed the same phenomenon "one afternoon". These observations imply a flight period of one to two hours and a flight distance of 5 to 12 km., whereas Macan's (1939) observations of corixids arriving between 16.00 and 17.00 hours is consistent with a flight distance of 20 to 25 km. This may well represent the maximum distance these species normally migrate. Leston and Gardner (1953) collecting corixids with ultra-violet light traps, operated from half an hour before dusk until midnight, found that 97% of their catch appeared in the traps within ¾ hour of sunset and no insects arrived after 1½ hours from sunset. These observations suggest that corixids are able to fly up to 9½ hours, during which period small corixids could fly about 50 km. and larger water bugs about 70 km. Even if it is assumed that emigration starts at 9.00 hours and the insects continue to fly until 23.00 hours, it would be reasonable to assume these insects have a maximum flight range of 65 to 90 km. per day.

Although migration normally begins in the absence of wind, migrating water bugs could be carried greater distances by air currents once they had risen some distance from the earth. As water bugs cannot survive in sea water for more than a few hours, the presence of these insects on volcanic oceanic islands can only be explained on the assumption they have been air-borne over the sea. The occurence of corixids on the Galapagos Islands, the Azores and the Hawaiin Islands, but not on the Tristan da Cunha group shows these insects can be carried, by wind, distances up to 4,000 km.

Migration Rates and Habitat

Although most species of water bugs are able to migrate, they do not do so with equal readiness, species characteristic of temporary habitats showing a greater readiness to fly than those species of more permanent habitats. This is well illustrated by the Corixidae, where species such as *C. nigrolineata* and *C. lateralis*, which are common in temporary habitats, show a greater readiness to migrate than semi-lacustrine species like *C. germari* or *C. carinata*. The causes of this phenomenon are being investigated.

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CONVECTIVE TRANSPORTATION OF CHORISTONEURA FUMIFERNANA (CLEM.)

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The distance which insects can cover by their own efforts is limited by the physiological limits of flight powers, by physical factors which modify these flight activities and by factors which prevent sustained orientation. In spite of these limitations, a large number of species do move long distances by virtue of sustained, well oriented flight. The example of long distance movement which I should like to discuss, however, is one in which the flight of the insects is of importance chiefly in providing for their launching under critical meteorological conditions. The actual long distance movement is the result of passive transportation.

The spruce budworm, *Choristoneura fumiferana* Clem. is an important pest of the boreal forests of North America. For many years, the insect has been studied in great detail, and extensive records of its population changes are on hand. One very striking characteristic of the population changes has been the occasional appearance of enormous numbers of adult moths in areas remote from the nearest known infestation. These flights have been recorded over a period of fifty years and over most of the known range of the insect. (The flight records are given by Johannsen, 1913, Tothill, 1922, Henson, 1951, and Greenbank, 1957.) More recently, Greenbank, 1957, has been able to detect the invasion of moths into areas of current infestation, and a number of such events have been recorded.

Adult *Choristoneura fumiferana* emerge from the pupae mostly in the foliage of the host trees. The males are strong flyers early in their adult lives. Females, on emergence, tend to be so heavy that they are unable to fly great distances. Following the deposition of the first egg masses, the females fly strongly. Spent females seldom fly (Wellington, 1948). Mating most frequently takes place in the host foliage near the emergence site of the female moths. The first egg clusters are deposited near the same sites.

The diurnal flight activity of male and partly spent female moths is very similar. During the morning, there is little flight. As the day passes and the temperature increases, occasional, rapid, direct flights between trees take place. On warm still days, these flights may be quite frequent and there may be considerable hovering near the foliage. Flights are suppressed by low temperatures, high winds, low evaporation rates and rainfall.

During the evening, as the light starts to decrease, large numbers of males and partly spent females rise above the trees in a sustained, hovering flight. This crepuscular activity lasts about an hour with a peak of about ten minutes. It ceases abruptly at dark. The insects are stimulated to fly by rapidly decreasing light levels. The number which fly at this time of day and the duration of their flights is increased by high temperatures, light winds and moderate evaporation rates. Rainfall, very low evaporation rates, low temperatures or the rise of the moon quickly suppress the evening flight.

Two important exceptions to this overall daily pattern may be seen. First, a sudden decrease in light intensity caused by a heavy cloud will be followed by a short-lived wave of flight during which a large number of moths rise above the trees in a burst of activity lasting only a few minutes. Secondly, a similar burst of activity is induced by the violent microbarographic disturbance associated with a convective storm. This microbarographic effect can be seen even though the storm does not pass directly over the area observed.

Examination of meterological records for the time of appearance of heavy invasions of *Choristoneura* show that in virtually all cases, the dispersal was associated with the

passage of a cold front. The distance from the nearest known source of insects to the area of deposition was as great as 250 miles (415 km.).

The mechanism appears to be a passive transportation by convective storms. When a cold front passes over an area of infestation, the line squall which lies in advance of the front causes sudden shading of the area. At the same time, there is extensive microbarographic activity. These two events cause a heavy flight of moths to rise above the trees. The insects are carried aloft by the foredraft which blows up into the leading edge of each convective cell.

Each cell of a line squall is made up of all the components of an airmass thunderstorm. There is a foredraft blowing up into the leading edge of the cloud, a main cell with a central downdraft, and secondary cells with their associated winds. Air speeds within the storm are very high: high enough to recirculate water and ice particles until they grow to a weight which carries them through the circulating systems. Insects blown into the storm would be circulated the same way as water drops, but as their falling speeds would be lower than those of rain drops, the insects would tend to stay aloft.

Deposition would always be expected to be patchy. Moths would be deposited either with rain, by the collapse of the convective cell or by being tossed out the top or sides of the storm. Deposition has most often been observed during the evening and early night hours when the activity of the convective cells would be decreasing with the nightly decline in instability.

The prediction of emigration and its trajectory and destination would appear to be possible by normal methods of synoptic climatology. The passage of an appropriate weather system, particularly during early evening, could be traced and the degree of instability during the night used as a basis to anticipate deposition. Detailed examination of the weather records for the periods just prior to known deposition shows that this approach holds promise. In all cases examined, it was possible to reconstruct a reasonable explanation of the insect's origin.

Judging from the speed of frontal movement and from the times at which deposition has been observed in the past, the most widespread launching takes place when frontal passage coincides with the normal evening flight. The transportation of moths by airmass thunderstorms has been suspected but not verified. Insects deposited are for the most part active and able to lay eggs. There is little reason to expect that being carried in a thunderstorm would damage them unless they were subjected to ice deposition (Wellington, 1945). The deposited insects tend to be predominantly female (Greenbank, 1957).

A number of insects other than the spruce budworm have been recovered under conditions which suggest that they have been transported in prefrontal storms. Greenbank (1957) reports that very heavy flights of forest tent caterpillar moths (*Malacosoma disstria* Hbn.) suddenly appeared in conjunction with a heavy deposition of *Choristoneura*. In this case, the launching conditions are not known.

By means of a simple but ingenious calculation which is based on the ratio of numbers of deposited eggs to females, Greenbank has been able to measure the effect of convective transportation on local populations (Greenbank, 1957). His conclusions were that moth invasions hasten the build up of local infestations and re-establish waning infestations. By the same token, emigration may reduce the rate of local build up. These effects may be very dramatic. As much as fifteen-fold excesses in egg populations have been recorded following a heavy deposition of moths. Factors of stand composition and local micrometeorology are critical in the initiation of outbreaks and in the local establishment of dispersing moths.

It is interesting to note that the convective transportation of *Choristoneura fumiferana* shows at least one characteristic which is rather unusual. Johnson (1960) cited a large

number of cases in which mass migrations represented the first adult flight or one very close to it. In the case in hand, the migration represents a flight following mating and some egg deposition. Greenbank's (1957) results confirm this earlier conclusion (Henson, 1950). He found rather less than a third of the original compliment of fertile eggs in a series of dispersed females. This suggests that the insects had been active for a considerable length of time before dispersal. The inability of fully gravid females to fly well and the sporadic timing of the frontal passages account for the situation as it is seen. I feel, however, that the fact that this case does not seem to conform to Johnson's general conclusion suggests one very good reason to regard passive transportation as distinct from the phenomenon of "migration".

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MIGRATION OF THE MONARCH BUTTERFLY (DANAUS PLEXIPPUS)

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Manuskript nicht eingelangt

ABSTRACT

Based upon the information obtained from a continentwide tagging programme, carried out for a period of eight years, the following conclusions have been reached: The fall flight is from north-east to south-west. The spring flight is from south-west to north-east. The migration extends from the breeding grounds in the northern parts of the United States and southern parts of Canada to the Gulf coast and Mexico. Western populations may over-winter in California on "butterfly trees" from San Francisco to 50 miles north of Los Angeles, or as free-flying individuals in Southern California, Mexico and the coast of the Gulf of Mexico. Fall flight commences during the last week of July, reaching maximum movement in September. Spring flight commences in late January with maximum movement in March. The fall flight is leisurely and feeding takes place daily. Specimens deprived of nourishment died in ten days—the reserve of fatty tissue was apparently not utilized. The spring flight is rapid and direct with no indication of feeding. Spring migrants were kept for 30 days without food. It was concluded that the fatty tissue is utilized during the spring migration at a time when flowering plants are not abundant and when it is necessary for the ovipositing female to reach the breeding grounds as soon as possible. Full results of this investigation, carried out over a period of seventeen years, are contained in a book "The Monarch Butterfly", University of Toronto Press, 1960.

THE MECHANISMS OF DESERT LOCUST SWARM MOVEMENTS AND THE MIGRATION OF INSECTS

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A swarm of locusts, comprising between 10^5 and 10^{10} individuals, represents a discrete population which is often morphometrically distinguishable from other swarms. Thus for example samples were taken for morphometric examination from each of three swarms of the Desert Locust (*Schistocerca gregaria* Forsk.), which successively passed within 40 km. of Wajir in Kenya between 7 and 14 February 1952. The three swarms, travelling in a generally westerly direction and some 100—300 km. apart, were among a number which had been produced in the Somali peninsula as a result of widespread breeding in late 1951, and the samples, which ranged in size from 80 to 629 locusts, showed a number of points of general morphometric resemblance. However, using the criteria provided by measurements of length of elytron (E) and hind femur (F), and of caput width (C), together with the ratios E/F and F/C, for both sexes separately, the three comparisons between swarms showed differences which were statistically highly significant ($P < 0.01$) in respect of 2, 2 and 6 of these 10 criteria. The three swarms, despite such similarity of origin and history, thus represented statistically distinct populations.

Such swarms, followed by aircraft, have been found to retain their identity during displacements of hundreds of kilometres over periods of weeks, maintaining their cohesion, by the active behaviour of the flying locusts as they reach the perimeter of the swarm, despite the disruptive effects of atmospheric turbulence (Rainey 1958). This has made it possible to study, in more than a hundred cases, the hour-to-hour and day-to-day displacements of individual swarms, in a manner which does not yet appear to have been possible for discrete populations of other insects. On 45 occasions so far, these hour-to-hour observations have established the direction of displacement of a whole swarm within 100 km. of a point at which instrumental observations of the corresponding wind were being made at the same time at the levels of the flying locusts. In half of these cases the direction of displacement of the swarm was within 10° of directly down the corresponding wind (the vectorial mean wind between the ground and the level of the topmost locusts); the biggest difference from directly down-wind movement so found was 35°; and these differences are likely to be within the limits of accuracy of the observations concerned. All these observations were made in winds light enough for an up-wind displacement of flying locusts to be physically possible; there was no question of these down-wind displacements being imposed upon the locusts by wind-speeds in excess of the flying-speeds which they individually exhibit.

This evidence is consistent with earlier findings on the mechanism of swarm movements (Rainey & Sayer 1953, Waloff 1958). Thus while groups of strikingly uniformly orientated flying locusts have invariable been noted in observations of flying swarms, systematic recording has shown each swarm to comprise numbers of such groups, with the widest possible diversity of orientation between different groups in the same swarm at the same time. In relation to direction of displacement, the orientation of the flying locusts in the swarm as a whole appears to be effectively random; and down-wind displacement of the swarm accordingly follows.

Some years ago it was pointed out that a down-wind displacement of swarms, on a geographical scale, must result, in general and on balance, in movement towards and with zones of convergent surface wind-flow, which are in general likely to be areas of

rainfall (Rainey 1951); and evidence continues to accumulate indicating that this may well be the key to the successfully nomadic continued existence of the Desert Locust, living in regions of scanty and erratic rainfall with an egg-stage entirely dependent on access to free soil water. The dynamics of the wind-systems involved in the production of rain are in fact such as to confer upon the flight habit, regardless of orientation, a substantial survival value for the fauna of arid regions.

The evidence now available on the mechanisms of movement and distribution of locust swarms thus emphasises the dominant role of wind in determining the direction, speed, route and destination of these population movements, and has not yet established a single case of "migration" of a swarm as a whole, in the sense of movement in a direction apparently under the control of the insects concerned (Williams 1930, 1958). Since locusts have in the past been regarded as among the most characteristically migratory of insects, these findings have suggested some re-examination of the evidence for migration in this sense in other insects.

The literature provides a number of records of other migrating insects suggesting a relationship with winds and weather comparable with that shown by the seasonal movements and distribution of locust swarms. For example, records of *Libythea* in western Africa, of the "flighting" of a number of species of Pieridae in southern India, and of Danaidae and several other families in Ceylon (in Williams, l.c.), all show a marked association with the particular times of year when the wind regime of the area concerned is dominated by the passage of the Intertropical Convergence Zone—reminiscent of the association which has been found between the Zone and locust swarms (Rainey 1951). Again, it has been suggested that migrating Lepidoptera characteristically fly lower than locusts, since up to 1951 there were only a dozen or so records of Lepidoptera migrating at heights of more than 30 m; but, with increasing opportunities for such observations from light aircraft, *Colias lesbia* F., for example, has been seen in South America flying in numbers at a height of 2000 m (Harriet 1955).

However, even casual and incidental personal observations on the common African migrant *Catopsilia florella* Fab. suggest a need for caution in generalising from the locust results. Thus for example in January 1950, near Cub-cub in northern Eritrea, Ato Adefris Bellehu (now Director-General of the Ethiopian Agricultural Department) and I chanced to observe a number of widely separated successive individuals of this Pierid, flying up one side of a roughly conical hill feature and down the other, remaining within a few metres of the ground, and giving the impression of maintaining a single, consistent track, without obvious relationship to the wind at the time; the butterflies appeared often to be out of sight, both of each other, and, after passing the summit, of their earlier path, whose line they appeared to maintain. Again, in July 1951, individuals flying across a Nairobi garden were noted as showing repeated small changes of orientation, giving a resultant impression of maintaining a relatively uniform track despite fluctuations of wind. I have not, myself, seen either of these types of behaviour in flying locusts—though this may perhaps be at least partly due to the difficulty of following, for more than a few seconds, the behaviour of one individual in a swarm; and overriding effects of mutual stimulation may also be involved.

But what was particularly lacking in these casual observations of *Catopsilia*, as in so many of the records of insect migration in the literature, was, of course, evidence of the corresponding displacement—if any—of the particular insect population as a whole; and experience has shown how the characteristic uniformity of orientation seen within a single group of flying locusts can suggest a wholly misleading inference as to the direction of displacement of the swarm as a whole. With locusts, the only type of observation which has so far been found to provide the evidence necessary to establish

the direction (and speed) of a population of flying insects, relative both to the ground and to the air through which they fly, has been successive determinations, from the air, of the position—and extent—of a particular swarm. Observations of this same type, on for example flights of *Catopsilia* as conspicuous as those seen in eastern Africa, should require no more than a few hours' flying by an appropriately interested observer in one of the now ubiquitous light aircraft. Such observations, together with the corresponding routine data on wind at the levels of the flying insects, and examined by the convenient graphical methods of elementary air navigation which have already provided evidence on the orientation behaviour not only of flying locusts but also of homing birds (Rainey 1960), would make it possible, for the first time, to estimate what contribution is in fact made to the displacement of such a population as a whole by the apparently purposeful behaviour which is so impressive to the observer on the ground.

It is, however, already clear that migration, in the commonly used entomological sense of displacement in a direction apparently under the control of the insects concerned, can play very little part in the biologically significant long-range displacements so characteristic of locust swarms; and, so far as I am aware, migration of a whole population, in the sense of this definition, has not yet been established in a single species of insect by evidence which, in the light of the experience provided by locusts, can now be regarded as adequate.

But I suggest that such a definition, insisting that the direction of displacement made good by a migrant must be "under the control" of the animal, is more a reflection of the characteristic and powerful impression made by the phenomenon on the observer, than of what we now know of the nature of the phenomenon itself. And, rather than restrict the term "migration" to a phenomenon not yet convincingly demonstrated to occur at all among insects, I suggest the alternative definition of migration as "seasonal displacement of population", which is consistent not only with what is now known of the mechanisms of migration of the most characteristically migrant of insects, but also with much of current ornithological usage.

Similarities between the pattern of migration of Desert locust swarms and the pattern of migration of some of the birds of the same region, together with recent evidence of the extent to which current weather can account for the day-to-day embroidery of the basic pattern of bird migration, suggest that the evolutionary origin of bird migration may perhaps have been in seasonal displacements of population, initially due to a meteorological mechanism similar to that of present-day locust migrations, and that the development of the striking powers of bird navigation may have been a refinement which came later.

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DISTRIBUTION OF THE DESERT LOCUST (SCHISTOCERCA GREGARIA FORSK.) AND ITS CHANGES IN TIME AND SPACE

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ABSTRACT

Cartographical analysis of historical material on the Desert Locust, assembled over thirty years, has helped to clarify the biogeography of these insects, shown by field studies to fly activity at all stages of adult life, and to move with the wind.

The species occurs over large parts of Africa and south-western Asia, with diverse climatic conditions, but with mostly poor and erratic or strictly seasonal rainfall, falling in alternate seasons in the tropical and subtropical parts of the areas. The occurrence of the Desert Locusts is similarly seasonal in each part of the area, and their breeding is closely linked up with the incidence of rains. As a rule new swarms emigrate soon after fledging, and often traverse thousands of miles before they reach the areas in which they breed. The direction in which the swarms move is dependent on the wind-fields in which they find themselves—and the intervals between successive generations and rates of development vary according to conditions in the transit and breeding areas.

The sizes and positions of the areas occupied by swarming populations change continuously, expanding and contracting with the seasons, and within the seasonal areas the extent and location of infestations varies from year to year, reflecting the variations in synoptic situations and rainfall distributions. Some parts of the area are, however, infested more frequently than others, and the highest infestation frequencies are observed in the areas where climatic conditions appear to be most favourable for rapid development. Short term fluctuations in the extent of affected areas are interspersed by longer-term recessions, which follow failure of breeding, but such periods may be relatively brief, and are characterised by the persistence of swarming populations.

It appears that the species is able to survive and to maintain its populations at a high level in spite of the unstable and often unfavourable environment, by adopting a highly mobile form of existence, by virtue of which it is able to utilise for breeding seasonally suitable conditions in widely separated geographical regions, and to reach frequently those parts of the distribution area in which breeding conditions are most favourable.

A FUNCTIONAL APPROACH TO INSECT MIGRATION AND DISPERSAL AND ITS BEARING ON FUTURE STUDY

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Students of migration and dispersal have hitherto been particularly preoccupied with the actual displacement process and with the sudden appearance of "mass migrations"—both where the insects appear to have control over a common direction of flight (as with many butterflies) and where they drift on the wind, like aphids.

Because the origin of such migrations is rarely recorded or even seen, migration has been detached from its context in terrestrial ecology. Definitions of migration, and approaches to its study, have therefore concentrated on qualities of flight during displacement—e.g. persistence, or a common orientation. For many entomologists a common flight direction is the main

evidence of a migration which is regarded as a fundamentally different phenomenon from flights in which insects drift, or disperse, in a so-called "passive" manner.

Thus controversy as to what is, or is not migration or dispersal has arisen; and like all events detached from their true context (in this case, the origin in a terrestrial population) mass migrations have had a certain mystery and some *special*, and indeed spectacular, qualities of flight have misleadingly been given *general* significance. This neglect of events at the start of migrations has stultified a functional, biological view of the process.

Many entomologists have felt that something has been wrong, but the controversy has never been fully resolved: indeed in this present symposium distinctions have been made between migration as distinct from dispersal flights. Kinds of flight during displacement are however very varied and to choose one or another, no matter how spectacular as a basis for a hard and fast general definition of migration leads to endless controversy. Thus provided an insect finds its host or habitat a movement of a few yards may be as effective as that of a few hundred miles: mass movements, though at the mercy of the wind, are usually considered migratory, for, as Dr. C. B. Williams (1958) recognised with the wind-borne Diamond-back moth, ". . . the large population that moves simultaneously indicates something of a migratory nature". However, numbers involved, distances travelled, and common orientation are all unsatisfactory criteria for what is or is not migration.

What then is the essential character of "migration" if it is not distance of displacement, common direction under the insect's control, or the mass effect so often associated with migration but which is obviously also too variable to be used in a definition?

A new view of migration and dispersal

The present view in this paper has developed from our work on aphids at Rothamsted and its similarity to the works of Provost, Nielsen and Haeger on the mosquito, *Aedes taeniorhyncus* and the butterfly *Ascia monuste*. Until five or six years ago it appears to have been assumed by many entomologists that the winged aphids produced on a crop (e.g. *Aphis fabae* on beans) stayed near the crop, flying about more or less numerously according to the weather and gradually dispersing, by accident, into the wind currents of the upper air.

We now know that the process of dispersal, or migration, is quite different. As soon as a winged aphid reaches flight maturity at the end of the teneral period immediately after emergence, it at once flies away permanently if light and temperature permit. The apparent urgency and irresistibility of flight and the positive phototaxis at this first take-off are remarkable. The insects put themselves deliberately, so to speak, at the mercy of the wind; not just a few by accident, but most by design. They do not return except inadvertently, and indeed the crop which produced them may itself soon die.

The exodus occurs whether the alatae population is large or small, and this behaviour of the alatae (as distinct from the production of alatae in the population) is not due to population pressure or current lack of food. It is the normal act of the new, individual adult.

Aphids are insects of the "aerial plankton". Many entomologists see them not as "true migrants" but as "passive drifters" similar to ballooning spiders. But as J. S. Kennedy (1951) pointed out long ago all flight is active: buoyancy is in fact due to active flight and not to forces acting on a large passive surface area. This however does not explain migration, for all flight is active. There is another most important point—namely that the migratory flight as we usually see it en masse in aphids is the exodus flight from the original breeding site by new adults or a continuation of it. Furthermore, a similar exodus is a general character of most so-called mass migrations in other insects which are commonly accepted as migrants whatever the individual flight characteristics or the relative degree of later, "non-appetential" flight may be. Let us examine this.

The start of a migration

In Dr. C. B. William's recent book "Insect Migration" there is a record of the start of a butterfly migration. This is Skertchly's description in 1879 of a simultaneous emergence followed in an hour or two by the mass departure of thousands of *Vanessa cardui*. About a score of other records exist however not only including Lepidoptera (see Nielsen 1958 and his paper in this Symposium), but also migrating Coleoptera, Odonata, Heteroptera, Ants, Termites, Homoptera and Diptera and I have recently given a list of these (Johnson 1960). This apparently irresistible exodus flight with its strong positive phototaxis, by new adults from the breeding site on the first flight, or one soon afterwards, characterizes migrants of many orders and I suggest that mass migrations, as well as others that pass unnoticed, start in this way.

This flight appears to be relatively undistracted by mating, feeding or oviposition (i.e. it is as Provost, 1957, pointed out for *Ae. taeniorhyncus*, "non-appetential") though its duration and orientation differ according to species and circumstance. It seems to be taken in the first place at least only to get away from the place of birth. This is, I suggest, how migrations begin. How they continue is another matter. If this view is accepted several things become clear:

(1) The difference between migratory and non-migratory insects is that migratory ones leave the breeding site on a relatively "non-appetential" flight soon after emergence: non-migratory ones behave differently. This, I suggest, is a better distinction than one usually implied in the controversial question "what is migration?" with its present emphasis on displacement characteristics.

(2) Common orientation, apparent control over direction, or persistence, so long used in attempts to define migration, offer no basis for a general definition because each is a special and variable quality of the species or circumstance. Flight may have a long duration, and common orientation as with many Lepidoptera, long duration but direction determined by the movements of air masses, as with locusts, it may last a few hours while the insects drift on the wind, as with aphids, or for a few minutes only as with ants and termites. It may or may not be repeated. Such variable qualities have no doubt been evolved in relation to survival of the particular species. But even with the butterflies, which show an apparent control over a common direction, compass diagrams usually show some flights in other directions too—and, such migrations appear also to be dispersive.

The aerial plankton is not necessarily to be seen as primarily composed of insects which have got there passively and by accident (although of course some do) but because of the flight habits at exodus, and especially the time of day when flight occurs; that they are mostly small insects is, I think, not so much because small insects are more buoyant and more easily blown off course than large ones but because they are in fact the commonest. As Taylor (1958) points out there is a "boundary layer" for insects between earth and upper air which varies with depth according to species and circumstance. Within this layer the insect can control its direction of flight: it is, as he says, the penetration of this layer which is the crucial act in deciding whether the insect will be blown with the wind, and many insects have made a habit of penetrating this layer (see Taylor 1960).

(3) The mass effect so commonly seen is due, not to a mood descending on a collection of variable aged adults, but to a simultaneous emergence followed by an exodus flight in the first place. I do not deny that some insects (e.g. locusts and many Lepidoptera) are stimulated to fly by others in flight. But I maintain that this is a secondary quality

and the primary one is that, taken individually, in leaving the breeding site on a more or less vigorous undistracted flight soon after emergence.

Simultaneous emergence and flight maturation usually occur daily during the rise and fall of a population at the source. Sudden isolated appearances of masses of a flying insect are caused by local changes in altitude or direction within this continuous process and not by a sudden onset of a "migratory instinct".

(4) The initial early flight of new-adults raises interesting physiological problems and for the first time indicates where opportunity for an experimental approach lies: we no longer have to wait for an unpredictable event in the field. There is a significant paper bearing on this by Rockstein (1959) in which a process of biochemical maturation (metachemogenesis) associated with the development of maximum flight capacity in young adults is outlined. Rockstein shows that in many insects maximum flight capacity is built up soon after emergence: enzyme systems controlling muscle contraction and the transmission of nervous impulses to the muscles become integrated and the gonad maturation and the deposition of the adult fat body controlled by the corpus alatum also plays a part.

(5) The evolutionary significance of migration seems to me to lie in two aspects.

(a) As Dr. Southwood has pointed out in this symposium, in the impermanent nature of the habitat, which makes it necessary for a population to move away from it.

(b) The type of displacement flight in relation to the needs of the particular species.

There is a wide range of flight achievement both from species to species and between the individuals of one species, and also in the relative proportions of any population which shows migratory behaviour. All these attributes are expressed collectively by similarly aged, newly emerged, adults and I imagine that it is during the teneral period or at its end that direction, duration or proportion migrating are determined.

(6) There remain the special flights from hibernation or aestivation sites. Are these continuations of a process already begun but temporarily in abeyance? Or are they a rebirth of a totally extinguished physiological state? The answer I think lies with the physiologists and in the relation of the sexual cycle to migratory flight activity.

Finally, I think the old controversy as to what is or is not migration or dispersal, active or passive, is now on the way to being resolved and, most important of all, that by recognising how, when and where mass dispersive movements start (and of which "migrations", as often understood, are a special category) an opportunity is afforded to make experimental and physiological as well as ecological and evolutionary approaches to the subject.

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CONTROL MECHANISM IN AERIAL DISPLACEMENT

J. S. KENNEDY

The earlier separation of long-range displacement into two categories, active in strong fliers and passive in weak ones, has broken down in face of mounting evidence that the long-range transporting vehicles for aphids and locusts alike is the atmospheric circulation. This use of the upper air as a vehicle greatly extends the possible range of dispersal of aphids, and leads desert Locust swarms automatically to areas favourable for breeding. Some of the translocatory functions that are carried out, and so directly controlled, by the insect's locomotor responses during short-range, low-level flights when the air is only the medium and not also the vehicle of travel, are thus taken over by the air in these long-range displacements. This important discovery has been interpreted to mean that such movements hardly qualify as migrations, on the grounds that behaviour plays little part, except in secondary features such as the maintenance of cohesion in locust swarm. However, a number of sensory mechanisms will be discussed whereby their long-range displacements are controlled by both aphids and locutsts. The control is indirect but nonetheless behavioural, through responses and changes of responsiveness that govern embarkment upon and disembarkment from the air vehicle. The mechanism of spatial re-distribution of these insects is analogous with what is called in the laboratory a kinesis, not a taxis. The notion that directed movements must be taxes has been the main obstacle to recognition of aerial transport as a mode of migration.

MIGRATION — AN EVOLUTIONARY NECESSITY FOR DENIZENS OF TEMPORARY HABITATS

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Animal movements can be divided into two types: dispersive (or migratory) and trivial; such a division has been made for mosquitos by Provost (1952). Dispersive movements are those that lead to a scattering of the population, an increase in the mean distance between the individuals, trivial movement (sensu Heape, 1931) does not have this effect. Dispersive movement also has certain definite behavioural characteristics many of which have been enumerated by Johnson (1960): it usually occurs early in the adult life, just after the end ot the teneral period; the animals mount to the top of the vegetation, face the sun and take off upwards into the air. With flying insects the flight is usually unidirectional often positively orientated towards the sun or sometimes into the wind and is well described for the majority of species by Kennedy's (1951) phrase "persistant locomotor activity". Trivial movements contrast with dispersive movements in that they generally occur later in the life of the adult, when it is sexually mature; the take-off is haphazard, frequently stimulated by changes in light or temperature, and often, rather than being positively phototactic, the animals will turn if the light intensity increases too much. Within a population trivial flights are multidirectional and will cease immediately on perceiving prey, food or a mate.

Trivial flight may be described in the terminology of the Lorenz-Tinbergen behaviour school as appertive movement with an urge for food, a mate or shelter; dispersive movement has no goal and if there is any urge, it is for locomotion. Perhaps more satisfactory descriptions are available in the terms of the Sherrington-Kennedy theory

(Kennedy, 1958): in trivial movements the flight reflex is allied to the feeding (or other) reflex and the thresholds for food, or other factors, are low. In dispersive movements the flight reflex[1] is antagonistic to the feeding reflex and the thresholds, except for flight, are high.

Most migrations are dispersive movements by a large number of individuals of a similar age following a mass emergence (Johnson, 1960); when such animals are large powerful fliers, such as Lepidoptera and Orthoptera, they appear to migrate actively, independently of wind. Several writers, including Williams (1958) and Urquhart (1960), have distinguished between such "active migration" and "passive migration" by smaller insects such as aphids which are completely at the mercy of the wind. This distinction is not upheld because all insects are influenced by the wind and almost all cases of both so-called passive and active migration stem from the same basic type of dispersive behaviour. Just occasionally an insect engaging in a trivial movement may be blown off course, such individuals, vagrants, may lead to the dispersal of the species.

Many animals that disperse fail to find a new habitat and die, dispersal is therefore an expensive process for the species and its evolutionary advantage has been disputed. One explanation is that dispersal is a safety valve, a method of getting rid of excess population, but as Williams (1930) pointed out it is difficult to envisage how a character could be developed in the course of evolution if every individual that possessed it failed to contribute to future generations. Furthermore if such a theory is correct dispersive movement should be positively correlated with population density or food shortage. The work of Davidson & Andrewartha (1948) on *Thrips imaginis* and Christenson & Foote (1960) on various Trypetid flies in Hawaii has shown in these instances that the level of dispersive activity is not related to population density. Even in cases where dispersive activity is a response to population density, as with alate aphids and the gregarious phase of locusts, supplies of food are still ample.

If the end of the process of dispersal, rather than its commencement, is studied, then it is seen that successful dispersal results in the colonisation by the species of new habitats. This, it is suggested, is the prime evolutionary advantage of dispersive movement or migration. If this is correct then within a taxon those species with the most temporary habitats would have a higher level of dispersive activity than those with the more permanent habitats.

Temporary habitats are dung, carrion, annual plants, perennial plants of seral vegetation and temporary ponds. Such habitats being early stages in the biological succession, are only in one locality for a limited period of time. The semi-arid areas of the world with their spasmodic rainfall producing at irregular intervals lush ephemeral vegetation for short periods are regarded as temporary habitats. Finally those animals that feed on the flowers and fruits of a large range of plants also have temporary habitats having to change their location at frequent intervals. It is postulated that to colonise such habitats successfully animals must evolve a high level of dispersive activity.

Permanent habitats are rivers, lakes and the perennial plants (including trees) of climax vegetation, such as salt marshes, heathlands, marshes and woodlands. Here the habitat only changes over a long period. When once established in such an area—unless threatened by food shortage, and there is little evidence of this being a limiting factor in any permanent habitats—it would be a definitive evolutionary disadvantage for the species to have a high level of dispersive activity.

[1] Dr. J. S. Kennedy has since pointed out to me that this is not a correct interpretation of the Sherrington-Kennedy theory; the distinction lies entirely in the low thresholds for vegetative stimuli during trivial movements and their high thresholds during dispersive (migratory movements.

A measure of the different levels of dispersive activity within a taxon can be obtained in several ways. With large insects a high level of dispersive activity manifests itself as migration; migrant and non-migrant species can be distinguished in the literature. Seventeen of the British species of Anisoptera are never migratory, these species breed in rivers, lakes, permanent ponds (e.g. bog pools) and but rarely in temporary ponds. All the six regular migrants breed in temporary ponds (e.g. gravel pits) and some of them in lakes and permanent ponds as well, but never in rivers. The four occasional migrants all breed in both sorts of pond. The relationship between habitat and migration is seen in detail in the genus *Libellula*; *L. fulva* Miller breeds in sluggish streams and is non-migratory; *L. depressa* L. and *L. quadrimaculata* L. breed in ponds and lakes and are migratory.

If the habitats of the migrant species amongst British Macrolepitoptera are listed, it is found that they have temporary habitats their larvae feeding on annuals or perennials of seral communities; there are but two exceptions, *Vanessa antiopa* and *Lithosia quadra*, and the evidence for migration in these species is equivocal. Whereas for the majority of migrant Lepidoptera, Britain is a peripheral area, the Mediterranean basin and Middle East are centres of distribution. Wiltshire (1940, 1946) has made a series of studies of the migratory Lepidoptera in these regions and he concluded that the migratory habitat was responsible for their abundance in this semi-arid area, enabling them to colonise and breed in a series of temporary habitats produced and eliminated in turn by the vagaries of the climate.

Migratory Orthoptera are commonly known as locusts; all locusts are associated with semi-arid areas and their movements are cyclic and seasonal (Waloff 1960), so that, although some swarms perish in the desert, the majority move from feeding ground to feeding ground. The desert locust, *Schistocerca gregaria* Fosk inhabitats the most arid environment and is the most mobile of all locusts; Uvarov (1957) concludes that "its nomadism is essential for its survival".

The migrations of the Heteropterans, species of *Dysdercus* are examples of a seed feeding insects with a wide range of host plants, various Malvales. These plants only bear seeds for a few weeks in the year; *Dysdercus* have no resting stage and thus migration from one host to another, as their seeds become available, is an integral part of their life cycle.

Various aphidophagous Syrphids and Coccinellids are the groups most frequently recorded as migrants in their respective order. It is not surprising that these predators, whose prey are migrants, should be migrants themselves. The same appears to be true of the fly, *Stomorhina lunata* (Fab.), a parasite of locusts (Greathead 1959).

Probably the most satisfactory method of comparing the levels of dispersive activity in different species is to sample the flying population by a random method, such as a suction trap, and contrast the composition of this catch with the comparative sizes of the actual populations of the various species in the vicinity, estimated by some other method. Southwood (1960) made such a comparison for the Heteroptera of a field margin; he found that although those species with annual host plants had small populations in the environs of the suction trap, large numbers were caught. In contrast species associated with grasses and certain perennials had large populations near the trap and yet they were seldom caught in flight. More extensive records of flying Hetroptera confirmed that a high level of dispersive activity is found in those species with temporary habitats (Southwood 1960).

Lewis (1961) has made a similar study on the Thysonoptera; he divided the species trapped into two abundance groups and within each group into those with permanent and those with temporary habitats. The mean catch per species was much higher for those species with temporary habitats than for those with permanent habitats (750 & 120 v. 0.5 & 14.4).

In some instances it may be possible, from a knowledge of the location of the trap, to assume that dwellers in permanent habitats will not be present in much smaller numbers than those from temporary ones. This is so with Southwood & Johnson's (1956) records of flying Coleoptera and those species with a high catch all have temporary habitats, such as compost, dung, carrion and logs.

From the definitions of dispersive and trivial movements already given, it is apparent that where sufficient information is available on the flight behaviour of an insect, the level of dispersive movement can be judged. Kennedy & Stroyan (1959) in their review of aphid biology stress the difference between the hovering flight of tree aphids (not dispersive) and the departure of host, alternating species which soar upwards away from the host and into air currents where they no longer have control of their flight and are dispersive over a wide area. Haine (1955) first noted differences in the take-off behaviour of these two groups.

Several authors have made measurements of the flight range of various Trypetid flies; species with a wide range of host plants, e.g. *Dacus dorsalis* Hendel have a flight range of many miles (Christenson & Foote 1960), whilst those with a limited host range, e.g. *Rhagoletis cingulata* (Loew) on Cherry and *R. completa* Cresson, on walnut, have mean flight ranges of under half a mile (Jones & Wallaxe, 1955; Barnes 1959).

Pseudoscorpiones are wingless, their only means of dispersal is phoresy; they attach themselves to the legs of various flies and Opiliones. Vachon (1947) has pointed out that those species that are phoretic live in compost and vegetable detritus, whilst those whose habitats are trees or the soil seldom, if ever, disperse.

In groups where some individuals or species are winged and some wingless, it can generally be assumed that the latter have a much lower level of dispersive activity than the former. Such a condition obtains in the aquatic Heteroptera and Coleoptera; the studies of Parshley (1929), Greensted (1939), Brown (1951), Fernando (1958) and others have shown that fully winged individuals disperse activity quickly colonising new habitats. Jackson (1952, 1956 a & b) has found that many species of aquatic Coleoptera are either wingless or lack flight muscles, the majority of these species live in rivers or streams and none in artificial ponds or gravel pits. Brinkhurst (1958) has pointed out how those Gerridae that are always apterous are riverine, whilst pond dwelling species are fully winged in at least one generation a year. Those British Lepidoptera with brachypterous females are aboreal.

It is claimed that this range of evidence, full details of which will be given elsewhere later*, confirms that in the course of evolution a low level of dispersive movement has been associated with the colonisation of permanent habitats and a high level closely correlated with the adoption of a temporary one. Habitat type has been the primary factor in the evolution of a high or low level of dispersive movement; its role as a mechanism of population regulation has been of secondary importance, but none-the-less dispersal plays an important part in the population dynamics of denizens of temporary habitats, which, because of the rapid and continuous change in the locations of their populations, often have high rates of reproduction, but lack a complex set of predators and parasites.

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SYMPOSIUM III

CHEMIE DER INSEKTEN
(Insect chimistry)

und

SYMPOSIUM IV

CHEMISCHE VERTEIDIGUNGS-MECHANISMEN BEI ARTHROPODEN
(Chemical defensive mechanisms in Arthropods)

sind als eigener Teil der „Verhandlungen“ Band III mit Unterstützung der italienischen Regierung in Pavia (Istituto di Entomologia Agraria dell'Università) erschienen.

SYMPOSIUM V

HOST-SEEKING BEHAVIOUR OF MOSQUITOES

Introductory speech by the chairman:

THE PLASTICITY OF RESPONSE PATTERNS IN HOST-SEEKING MOSQUITOES

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Ethologically speaking bloodsucking, especially engorging on the host, can be considered as the consummatory act of the feeding behaviour. It doubtlessly leaves the mosquito in a state of quiescence, as may be clear from observations in the laboratory and in the field.

In nature nowadays this state even arouses a tremendous amount of interest, because it immediately bears to problems of the fight against mosquitoes. And the fact that bloodsucking is a consummatory act also underlies modern calculation of transmission intensity of pathogenic organisms. In that sort of calculation, indeed, we take for granted that a mosquito, when engorged, does not try to get the next bloodmeal before the last one is digested or a batch of eggs is deposited.

Meanwhile there are laboratory observations which do not seem to support this view: it is often seen as a proof of the avidity of *A. gambiae* that it will probe again and again even when engorged.

At this point we should of course immediately realize that what happens in a laboratory does not necessarily happen in nature, but.....it may happen. We are often in the unfortunate position that in order to understand behaviour in nature we have to resort to laboratory investigations. These may yield quite reliable results in the case of fishes, very often decidedly not in the case of flying insects as mosquitoes. Although there are many other reasons, for this one reason it is already not surprising that so many inconsistencies exist in literature regarding feeding behaviour of mosquitoes. Though in the last years field observations have come to give us good hints as to what mechanisms actually play a role in the host-seeking of a mosquito, it is unfortunately often difficult to link them up with results of laboratory research.

One reason at least for these discrepancies is, as it seems to me, the plasticity of the mosquito's behaviour.

All of us know, for instance, that in nature mosquitoes do penetrate human habitations from a rather great distance to suck blood, but these same mosquitoes readily take sugar water when maintained in a cage, say for reasons of salivary gland dissection.

It is often noticed that remarkably few mosquitoes bite near the breeding places, thus suggesting the necessity of a prebiting dispersion flight (for which, in some species

at least, there seems to be good evidence). But in the laboratory many species will bite without preliminary flying within 24 hours after emerging from the pupae, much sooner than is mostly observed in nature.

Bloodsucking decidedly is the target of female mosquitoes, but it is still poorly understood why most investigators had difficulties in making mosquitoes react to whole blood. This, on the other hand, suggests a certain rigidity of this last part of the host-seeking behaviour pattern. It is important to notice that blood is often readily taken through artificial or natural membranes. We succeeded also to get sharp responses of *A. atroparvus* in the olfactometer, in which one air-current had passed through defibrinated blood, both currents being equal as to CO_2, temperature and humidity.

Heat is an important factor, inducing many mosquitoes immediately to alight and to probe and it was concluded by Dethier in laboratory experiments to be the primary factor in the bloodsucking of *Glossina.* Nobody, however, will deny that in nature warmth, perhaps more exactly a temperature-gradient, would be a very poor factor in guiding the mosquito to its host! It is even justified to say that in mosquitoes as well as in *Glossina* many other stimuli are necessary for them to obtain their bloodmeal.

Much work has been devoted to the study of various chemical substances as attractants of mosquitoes. This Symposium surely will reflect the activities in this field. It is to be hoped that many of the existing inconsistencies can be reconciled by full discussion on details of the test-conditions. It is perhaps amazing that mosquitoes seem to like as much the worn wool socks of Mr H. or the ditto nylon of Mr K. as more selected substances as amino acids. We are still in doubt whether in nature there is a broad chemotaxis or a specialized one, whether the chemotaxis is a distant working mechanism in so-called precision guidance or only efficient in the vicinity of the host. Evidently mosquitoes need it to locate their host in nature.

Upwind orientation, necessarily connected with it and splendedly explained by Kennedy 20 years ago, does in fact exist in nature, and we were able to show in Africa that the requirements to it were exactly fulfilled. Even so, our experiments with rotary traps did not yield evidence for concentration of many species of Anophelines near houses.

We caught about equal numbers at 50 m and at 250 m distance from a village edge. This is quite compatible with Colless' conclusion regarding *Culex annulus*, based on mathematical consideration, that these mosquitoes arrive in the vicinity of their host by a process of random wandering.

It is here, that I should like to call your attention to the necessity to discern between attracting and activating substances. I am not convinced that mosquitoes start their search for a host without any extrinsic chemical stimulus. May be that the large number of sources from which smells can be derived, such as sweat, sebaceous glands and blood, each act at specific moments in the process of host finding.

Some factors, although generally attractive, seem to be capable to upset orientation. Thus Brown found no attraction by CO_2 in very wet air, and nor did we in some series of olfactometer tests. He also found no attraction when sweat secretion was too strong. Very recently Mr. Gerold found in our laboratory a remarkable effect of wetting the gauze through which Anopheline mosquitoes were allowed to feed on the shaven skin of a Guinea pig. Nearly all of any lot engorged through a dry cloth, just the opposite occurred with a wet one!

Eventually some remarks on cyclical activity. No mosquito will feed at any moment, even when her stomach is empty. But the clear nycthemeral and gonotrophic cycles observed in nature are by no means essential in the capacity of a mosquito to respond to a host.

These cycles certainly have intrinsic components, but they are largely governed by environmental factors as can be demonstrated easily in the laboratory.

STUDIES OF HOST-SEEKING BEHAVIOUR OF ANOPHELES ALBIMANUS

HAROLD TRAPIDO

The Virus Research Centre (jointly maintained by the Rockefeller Foundation and the Indian Council of Medical Research)

Manuskript nicht eingelangt

ABSTRACT

The host-seeking behaviour of *Anopheles albimanus* and several other concurrently biting mosquitoes was studied on the Chagres River in Panama. In the case of *Anopheles albimanus* it was found that in erect human subjects the bulk of the biting was on the lower extremities. In more than a thousand bites recorded there was not a single bite on the head while the feet, which comprise approximately the same surface area, sustained almost half the total bites recorded. Disproportionate biting on the lower portion of the body, although not so extreme, was also recorded in the case of supine human subjects. Inverted subjects, however, were bitten primarily about the head and upper extremities which were in this case in close proximity to the ground. On the other hand it was found that when the subject was placed directly above another, bites were sustained on the feet of the individual essentially standing on the head of another whose head did not experience bites.

These observations together with others will be discussed in terms of the cues used by mosquitoes in determining where on a host they seek to engorge.

DISCUSSION

HECHT: When baiting *Haemagogus* we had to wait perhaps twenty minutes before the first mosquito arrived; then others followed, perhaps 1—2 mosquitoes each minute for half an hour or so.

When baiting *A. albimanus* we had the experience, that it is of no value to undress; they will not bite much on the trunk, but they bite on the ankles or sometimes on the head or neck; this remains true when the bait is a person lying horizontally. But these observations were merely casual by-products and were not made in a satisfactorily systematic manner.

REID: It may be added that my colleague Wharton has made similar observations on the location of bites on the human body. *Mansonia longipalpis* bit mainly on the legs and feet whether the bait was standing or lying, while *Aedes albopictus*, which was also present, bit mainly on the trunk and head.

SOME PITFALLS IN THE INVESTIGATION OF THE HOST SEEKING BEHAVIOR OF MOSQUITOES AS GLEANED FROM FIELD AND LABORATORY STUDIES IN THE ORIENT

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During the past decade in a series of studies in Japan, Malaya and Pakistan, it was found that there are numerous shortcomings or pitfalls in the methods commonly used for investigating the host seeking behavior of mosquitoes and related blood-sucking flies. In several instances, we have fallen into the very traps which we will describe.

If one is to judge from the literature, the most common pitfalls are to be found in the use of the precipitin reaction for the determination of the host source of mosquito blood meals. Most entomologists are poorly informed on the subject of serology and consequently are ill prepared to evaluate the reliability of antisera. Some antisera are not sufficiently specific at the time of preparation to be relied upon. Others, particularly when bacterial contamination occurs, rapidly lose specificity. It is therefore necessary to check all reagents for the homologous titer and to check for cross reactions not only at the time of preparation, but at monthly or bimonthly intervals during the period of use. The entomologist should enlist the services of a competent serologist if he is unable to undertake this himself.

Failure to collect engorged mosquitoes in a manner which ensures a random, unbiased sample, is probably the commonest shortcoming in precipitin test surveys intended to determine host species spectra. The literature is replete with papers giving little or no information on where and how engorged mosquitoes were collected. Data presented in this manner are uninterpretable. Engorged mosquitoes collected in stables almost invariably contain equine blood and similarly mosquitoes found engorged in pig pens will almost always contain pig blood. Occasionally, as in Tokyo in 1950, when we found 60 per cent of the *Culex pipiens pallens* collected in houses to contain avian blood, exceptions to this rule will be found, but generally, freshly engorged mosquitoes found resting in an animal habitat will be filled with the blood of the inhabitant. If one seeks information on the spectrum of host species, engorged specimens must be collected from a wide variety of habitats in a carefully planned or in an unbiased random fashion. Hand collection of resting specimens almost invariably introduces bias. Perhaps the most satisfactory approach is to employ light traps or to undertake sweeping of vegetation in an area divided into a number of quadrats.

Another device commonly used for the study of the host tropisms of mosquitoes is the animal baited trap. While perhaps entirely reliable in a situation where mosquito species readily enter structures, our experience in Malaya indicated that this was not always the case. We were interested in determining whether or not two species, *Culex gelidus* and *Culex tritaeniorhynchus*, preferred cattle over pigs. In a study which extended over 8 months, we concurrently operated two identical traps, both at the same location, one baited with cows, the other with pigs. Each trap was operated on 57 nights. However, to check on the validity of our results, we also undertook concurrent animal-biting collections from both cattle and pigs at the same location. Animals used in bait traps and in biting collections were rotated to eliminate the bias resulting from variation among individuals. The results are shown in table 1 (slide). It was found that by animal bait trap collection both species showed a slight preference for cattle over pigs, while by direct hand collection of mosquitoes feeding on animals, *Culex gelidus* showed no preference for either host and *Culex tritaeniorhynchus* demonstrated a marked preference for cattle. Further, when we listed the order of frequency of the three commonest species collected in pig-baited traps, it was the exact inverse of the order of frequency obtained in pig-biting hand collections.

Much has been written concerning the necessity of equating the size or surface areas of animals when comparisons are being made of their attractiveness to mosquitoes. Perhaps, the most interesting work in this respect has been that of Dow, Reeves and Bellamy (1957) who found that comparable numbers of *Culex tarsalis* were attracted to birds of the same size whether of the same or different species, while the number attracted to birds of different size was directly proportional to their size. Engorgement rates, however, were found to be independent of the size of the bird, and varied for different bird species and for different individuals of the same species. It is obvious then that in the operation of animal baited traps, not only must one identify and count

Table 1

Comparison of concurrent animal bait trap collections and animal biting collections, Serdang, Malaya, September 1954—May 1955

Bait trap collections

Species	Average collection		Preference for cow over pig
	Pig bait	Cow bait	
C. gelidus	8	17	2×
C. tritaeniorhynchus	12	30	2.5×

Biting collections

Species	Average collection		Preference for cow over pig
	Pig bait	Cow bait	
C. gelidus	81	85	—
C. tritaeniorhynchus	23	136	6×

the catch, but engorgement rates must also be determined. It is of interest to note also that in 1951 we tested a series of almost 2000 engorged mosquitoes taken from traps variously baited with horses, pigs and birds and found that 5 per cent did not contain blood of the bait animal.

In both field and laboratory studies, we have found that mosquitoes commonly showed a marked preference for individual animals or people and that the magnitude of such differences frequently exceeded preferential differences in host species. These most certainly are not new observations and yet in face of this, many workers still fail to employ animals in rotation and to make use of the weighted mean or average.

Still other pitfalls manifest themselves in studies on host seeking behavior of mosquitoes. While we have not commonly encountered the problem in Japan or Malaya, many species in Africa and elsewhere have been found to manifest cyclic diurnal activity and therefore in such areas 24-hour catches must be employed if serious errors of interpretation are to be avoided. In heavily forested areas, the vertical stratification of mosquito faunas has been well established and workers should be aware of the danger of only undertaking host studies on the forest floor level.

In many respects, data collected in field studies are subject to greater error of measurement than are data collected in the laboratory, where variables are more readily controlled, and where the activities of technicians can be more rigidly supervised. The field worker therefore must scrutinize and evaluate field data in a critical manner before arriving at conclusions. We should like to illustrate this point with data we obtained in Malaya during 1954—1955. During this period, we were collecting information on the seasonal abundance of several Culicine species at a rubber plantation called Seaport Estate, located near Kuala Lumpur. To determine the validity of our sampling, two collection methods were routinely employed: hand collection of mosquitoes feeding on cattle and hand collection of mosquitoes feeding on humans. The results obtained for *Culex tritaeniorhynchus* are shown in Figure 1 (Slide). The most striking feature of these data was the discrepancy in the configuration of the two population index curves. One very plausible explanation would have been that we were dealing with two distinct populations of *Culex tritaeniorhynchus*, one a population which preferentially fed on cattle, the other a population showing a marked preference for man. The discrepancy in the two curves during the latter half of the period, might, on the other hand, be attributed to laxness on the part of the collectors undertaking the human-biting

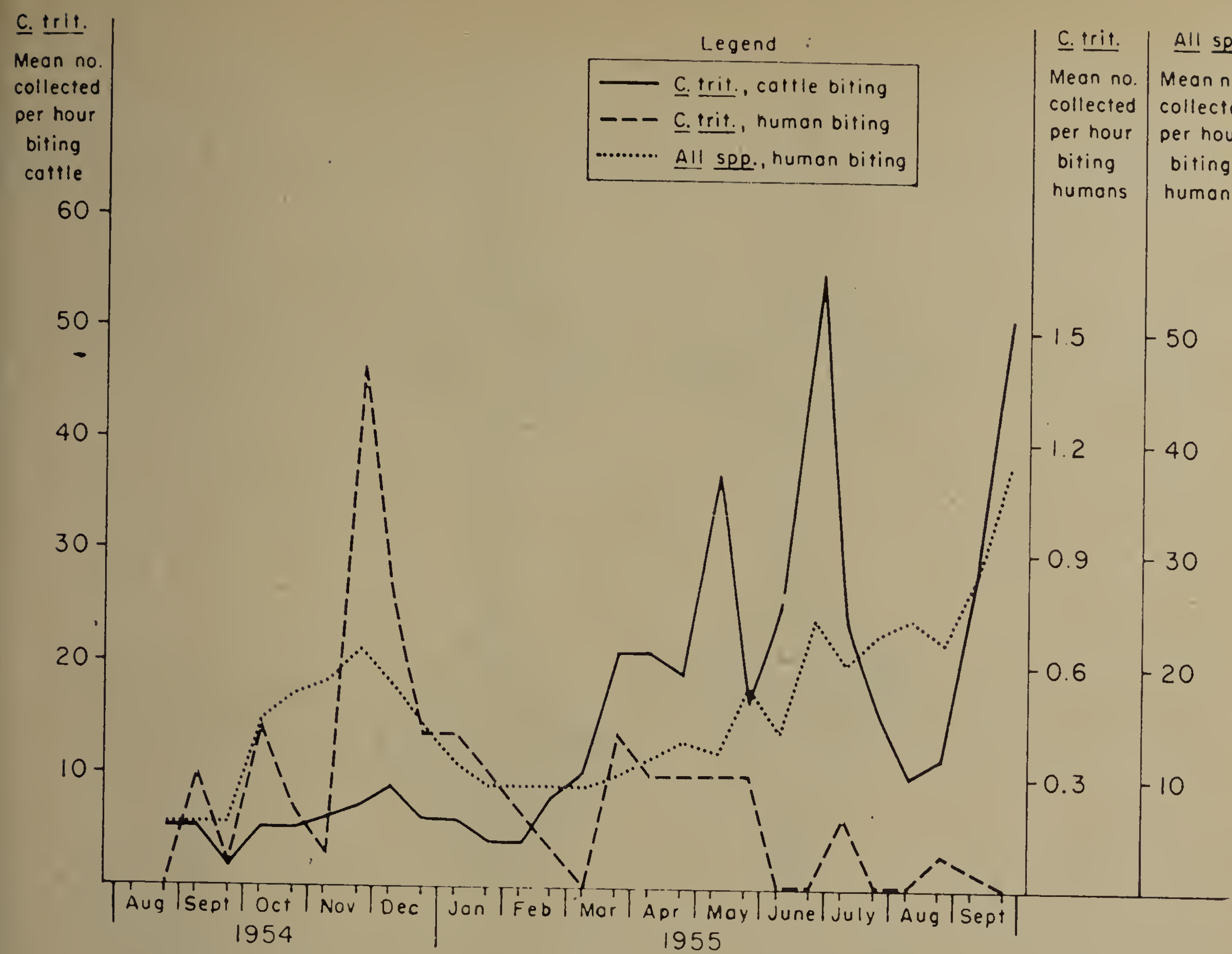

Fig. 1. *Culex tritaeniorhynchus* collections at Seaport Estate, Malaya.

collections. However, examination of the curve for human-biting collection of all (total) species, indicates that while collections of *C. tritaeniorhynchus* were falling off sharply, the total mosquito catch in these collections was rising rapidly. Does this prove the hypothesis of two distinct populations? We think not. Similar collections were made during this period in three other localities near Kuala Lumpur. At no other locality did we observe this marked discrepancy in the population index curves for *C. tritaeniorhynchus* obtained by the two methods of collection. Further, at Seaport Estate the picture we obtained in collections of *Culex gelidus* virtually duplicated that of *C. tritaeniorhynchus*, but this did not occur in the three other collection sites. We have concluded that there is an unidentifiable fault in our data from Seaport Estate, probably one of human origin.

It is hoped that these few examples of the pitfalls in the methodology currently employed in the studies of the host seeking behavior of mosquitoes will assist other workers in avoiding them.

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DISCUSSION

CORBET: I should like to comment on the speaker's suggestion that light-traps might be used as a host-independent method for collecting engorged mosquitoes for precipitin assays of blood-meal sources. We have been running mercury-vapour light-traps in forest in Uganda, and have found that only a very small proportion (less than 5%) of the female mosquitoes contained blood. It would seem then, that very few mosquitoes are active enough when engorged to be caught in light-traps. I should be interested to hear whether the workers using light-traps have encountered similar phenomena.

BARNETT: In response to the question on whether or not light traps attract engorged mosquitoes, it was stated that ordinarily light trap catches do not contain appreciable numbers of engorged mosquitoes, except when light traps are operated directly within animal shelters.

VARMA: I would like to ask Col. Barnett whether the attractiveness of birds to mosquitoes has anything to do with the amount of CO_2 produced by the animals, i.e. whether a larger animal would attract the mosquitoes by virtue of the larger amount of CO_2 given off, rather than by size.

REID: Dow, Reeves and Bellamy found that the number of mosquitoes attracted to birds was independent of the species of bird, but dependent on size. However, the birds were in cages rotated about a common axis, and this may have mixed up the specific odours of the different birds; thus accounting for the lack of a bait-species effect in the number of mosquitoes attracted.

AN EFFECT OF HOST DENSITY ON THE FLIGHT MOVEMENTS OF ANOPHELES GAMBIAE

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Experimental work on flower visiting insects has shown that it is necessary to distinguish between long range and short range orientation towards food sources (Dethier, 1953). Although there has been little investigation of the former type of reaction in blood sucking insects, the same undoubtedly holds true for them. It is suggested that the experiments described here provide an example of one set of circumstances in which this factor appeared to affect the movements of a mosquito population. Long range orientation is used here in the sense of Laarman (1955), who defined it as the phase of orientation leading to the arrival of the insect in the vicinity of the host.

I have recently made a study of the dispersion of *Anopheles gambiae* Giles in a coastal region of Tanganyika by means of marking and release experiments. The mosquitoes were reared in the insectary and were labelled either by the topical application of paint or by the use of radioisotopes. The present paper deals with the dispersion of mosquitoes recaptured during the first two days after release from two contrasted release points, a description of which follows.

The terrain chosen for the experiments was generally hilly, with small clusters of huts scattered throughout the area on the higher ground, a few of these being sufficiently large to warrant the designation of "village" (up to 50 houses). The prevailing aspect of the area is that of well vegetated, densely populated, foothill country. The two release points were approximately one mile apart, one of them being situated on the edge of a sizable village, the other in open country. Within a quarter-mile radius of the former there were three villages comprising a total of 80 houses, while near the latter there was but a single village of 15 houses, and even up to a distance of a half-mile there were only a few isolated groups totalling a further 24 houses. Mosquitoes were collected by means of routine spray catches in houses in the daytime. Throughout most of the area catches were made on a strictly regular basis in one house in five once a week, but in one sector this was reduced to once in two weeks. Catches were extended out to one and a quarter miles in every direction from the first release point, in the populous section of the district, and up to two and a half miles, in one direction only, from the second release point. The releases were carried out partly in small

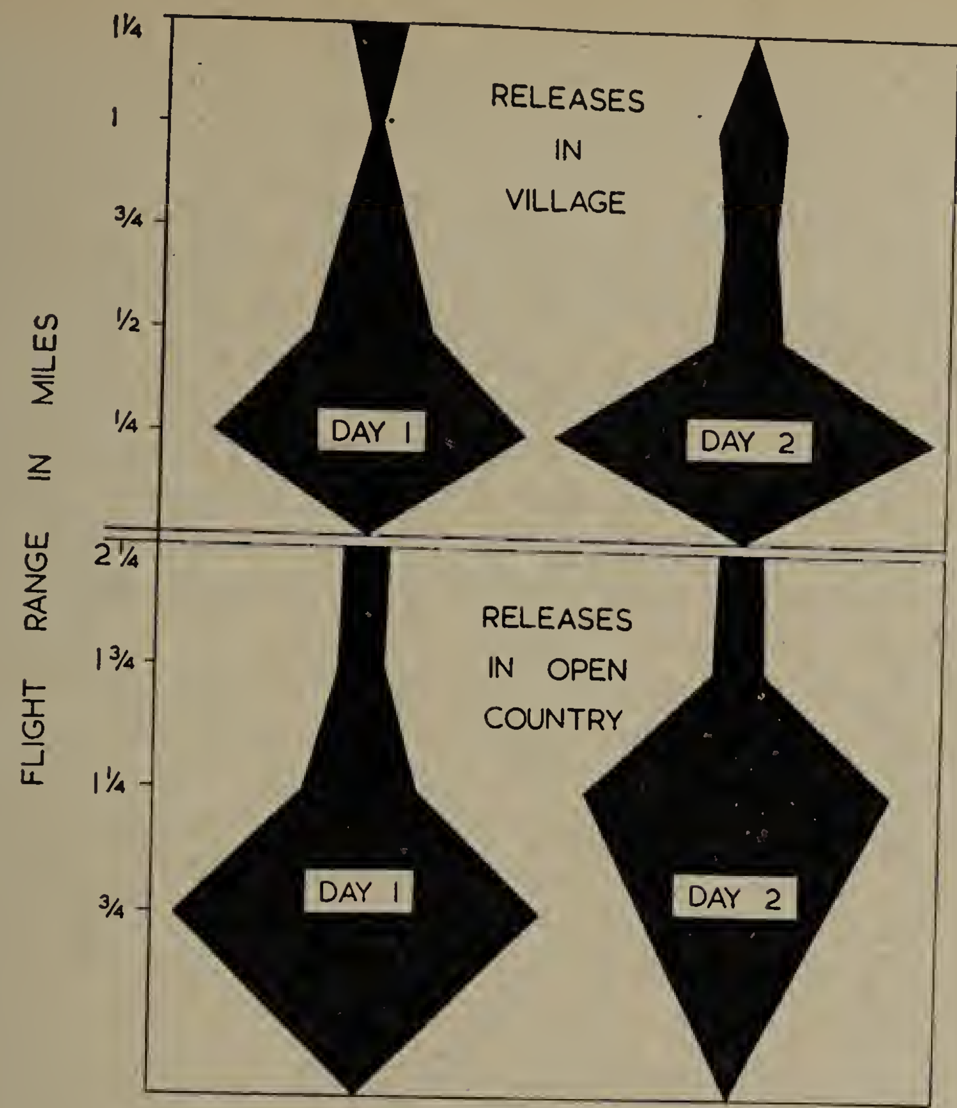

Fig. 1.

numbers over many months, and partly as a few large scale experiments, and the results shown may be taken as representing an average picture of dispersion over the whole period.

The mosquitoes were transported into the field in the late morning and their departure from the release point took place the same evening, an activity that was witnessed on a number of occasions. Figure 1 shows the dispersion of recaptured mosquitoes during the first two days after release. The figures relate to freshly fed females only, caught in houses in the day time and after correction for the chance of recapture in the different sectors and ranges. The diagrams show the initial concentration round the release point in both series and, in the case of those released in the populous sector, the essentially unchanged distribution of mosquitoes during the second 24 hours. On the other hand, the initial spread from the release points in open country appears to have continued during the second night, so as to give a very different picture of dispersal when compared with the first night's flight. This figure was biassed to some extent by the paucity of catching points in the thinly populated sector and to consequent difficulties in randomising catches in its vicinity. Nevertheless, even when allowance has been made for the probable effect of this factor, the differences between the two still remain considerable and warrant discussion in terms of host seeking responses.

Let us consider the various phases of activity of the mosquitoes. They were released in the day time, when a half to two and a half days old, in an empty hut or well shaded artificial outside shelter. At dusk a mass exodus took place under the combined influence of waning light and the normal endogenous rhythm of activity. Previous work in the area had shown that nearly all female *A. gambiae* take two blood meals during the course of the first gonotrophic cycle, that these are usually taken on two consecutive nights, and that mating may occur before or after the first blood meal. This preliminary blood meal appears to replace to a large extent the plant juice feed taken by many

Culicines, and perhaps by some Anopheles, during the first day or two of adult life. One is not justified in assuming that all the released mosquitoes behaved in exactly this way, since most of them had imbibed sugar water before release. However, we are only concerned with those caught freshly fed during the first two days after release. And if, as shown below, there is no evidence of any form of preliminary migratory flight, it may be assumed that their flight movements during this period were largely motivated by hunger. That being so, what are the possible alternative flight patterns that one might expect to find, and what light do the results shed on host seeking in this species?

If one postulates that flight is at random up to the point at which the host is detected, then the pattern of dispersion will depend on the effectiveness of the mosquitoes' receptors and on the density and distribution of hosts. As already explained, the area used for experiments was well populated; and the villages, although very variable in size, were in general fairly evenly distributed over the countryside. So the main factors influencing flignt range will be the density of houses per unit area and the powers of perception of the insect. If it can detect the host from a considerable distance, or in other words if the concentration of attractant necessary for orientation is relatively low, the actual numbers of houses in an area will be less important, since the mosquitoes will find them regardless of whether the settlement is small or large. On the other hand, if the host is only located at fairly close range, small settlements may be missed by a proportion of mosquitoes and the flight range of the population as a whole will be correspondingly greater.

Turning to the data shown in the accompanying figure, the catches from the populous release point illustrate the failure of most of the mosquitoes to disperse much beyond the inner circle of villages during the first two host seeking flights. This confirms the absence of any initial migratory flight in the majority, but it sheds little light on the question of host detection. It is of interest, however, that despite an abundance of food sources in the immediate surroundings of the release point a small number of females continued their flight to the limit of the recapture area and doubtless beyond. Whether this represents a failure of host detection or a delay in development of receptiveness to the stimulus cannot be decided.

In the case of those released in open country the initial spread is greater, as would be expected, owing to the paucity of houses in its vicinity. This spread is, however, more marked by the second day. This suggests that during the second night the movements of the mosquitoes at dusk and the early part of the night took them beyond the influence of the small villages in which they had fed or rested the first night. Such a conclusion gives general support to the view that long range orientation operates over a relatively short distance, although one cannot, from these data, express the effect in a quantitative manner. What is clearly shown is that, under certain circumstances, the density, as well as the distribution of the host, can have an important effect on the dispersion of mosquitoes. The greater the concentration of hosts in a particular area, the greater will the proportion of females be in which further flight is inhibited and a different activity pattern released. Thus mosquitoes will tend to be "captured" by populous localities, and it is probably this factor, in combination with the distribution of breeding sites, that determines the flight range of many tropical species.

A different view of the subject is provided by Colless (1959) in Singapore who, from the results of catches made in open net-traps, presented evidence of large numbers of unfed mosquitoes, many of which must have been hungry, entering an open mosquito-net trap erected outside. Since the net was unbaited and the opening faced away from the nearest breeding sites and towards the source of blood, Colless came to the conclusion that the mosquitoes had more or less blundered into it. This would suggest that flight

was random almost until they were in contact with the bait, or, in other words, that long range orientation scarcely existed. It is hard to fit this finding into the generally accepted picture of host-seeking, and further exploration of this method of trapping could be most enlightening.

In conclusion, it may be worth while considering two possible methods for assessing the distance over which long range orientation is effective. One could, for instance, present baits at increasing distances between each other until no further increase in catch occurred. Since the direction of approach of the mosquitoes would be unpredictable, the baits would have to be disposed radially. So far as I am aware, this precaution has not been observed in previous experiments, which have usually been designed for other purposes. But one notes the results of Reeves (1953) who, using CO_2 baited traps, found there was some interaction between them when set up at a distance of 40 feet apart. A second possible method would involve the devising of an alternative chamber, which would be set up at right angles to a gentle prevailing wind in an area uninhabited by suitable hosts. Test mosquitoes would be introduced into the down-wind compartment of the cage, and a bait then presented at decreasing distances up-wind from it until a response could be elicited in the form of a movement of mosquitoes from the down- to the up-wind chamber. The technical difficulties of such a method might, however, be considerable. Nevertheless, the problem is of more than academic interest and warrants closer study.

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DISCUSSION

TRAPIDO: Could the speaker please show a detailed map of the experimental area? It seems to me that the results indicate random dispersion rather than orientated flight.

GILLIES: I do not have a detailed map with me, but one will be published together with the full results of the experiments.

To clarify these results further, it is suggested that there are three possible mechanisms of dispersion—long range orientation operating over great distances; long range orientation effected over relatively short distances; lastly, purely random flight. The present data clearly rule out orientation over great distances, since otherwise the mosquitoes released in open country would not have dispersed widely on the second night. Of the other two mechanisms, purely random flight seems improbable in view of the very small proportion of the total area taken up by habitations. Indeed, the density of the houses in the district as a whole is so low in relation to the possible flight movements of mosquitoes, that if it were a question of simply flying at random until they more or less bumped into houses, the great majority of mosquitoes would never find a host at all until they had flown very great distances. Consequently orientation effected over short distances seems to be the most likely mechanism to result in the type of distribution described in this paper. Such a mechanism would, of course, be more effective if host searching was conducted as a group activity, as suggested by Dr. Trapido.

MATTINGLY: With reference to Dr. Gillies' contribution, it may be of interest to ask oneself to what extent the biting cycle of this species is a reflection of general flight activity rather than of biting activity per se. It would seem that in all probability the so-called biting curve is the resultant of the combination of a true biting curve and a flight activity curve. Given a sufficiently large number of catches there should be a progressive approximation to the ideal situation in which there is a uniform distribution of mosquitoes around the bait, the size of the population increasing as the square of the distance from the bait. The flight activity component of the biting curve will thereforebe a hollow curve with slope increasing progres-

sively in the proportions 1:4:9:16——:n^2. The mathematics of this situation were considered by Colless and, independently, by certain workers interested in light trap catches both arriving at similar conclusions. It is particularly interesting that the biting curve of *A. gambiae* is not, in fact, a hollow curve. It is a convex curve and this is, perhaps, its most enigmatic feature. In the ideal situation here envisaged the shape of the flight activity component of the biting curve will not differ significantly in conditions of directed as opposed to random flight but it seems worth asking whether it may not be otherwise in the experimental situation created by Dr. Gillies.

LAIRD: *Aedes polynesiensis* population baseline estimates in the Jokelau Islands (Southb Pacific) in 1958, by Dr. D. H. Colless and myself, and rechecks earlier this year, included adult catches based on 15-minutes collections from human volunteers (the collector himself being adequately treated with repellant so as not to confuse the count). On each occassion the collector and his accompanying bait walked along the lagoon beach, where *A. polynesiensis* seldom ventures, and then pushed through undergrowth to collecting sites (most of them under a coconut or Pandanus canopy).

Dividing the count into five-minute sections, it was found that in most cases the total felt off sharply from the first five-minute section to the second and from the second to the third. Fifteen minutes usually sufficed to exhaust or all but exhaust the blood-seecking *A. polynesiensis* ranging within the general area of the count or brought into it during the walk from the beach.

ATTRACTANT VAPOURS FOR AEDES MOSQUITOES

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In a report on the factors which attract mosquitoes to humans, communicated to the Tenth International Congress (Brown, 1958), our results obtained from *Aedes aegypti* were inconclusive on three points. First, hands of individuals that transpired more moisture were less attractive, although the reverse had been found with moistened inanimate objects. Second, although armpit sweat was attractive, forehead sweat was not. Third, although it was shown that beef blood contained an attractive factor besides carbon dioxide, its existence could not be separately demonstrated.

Since that time, Rahm (1956) has reported that moistening the arm increased its attractiveness, and that the hand is more attractive than the arm, emitting as it does much more water-vapour. Later, Rahm (1957a) showed that sweat from the hand was significantly attractive to *Ae. aegypti.* However, the odour volatilized from the hand was so much more attractive that sweat normally did not enter as a factor.

Whereas female *Ae. aegypti* uniformly respond to the human hand and arm on successive days, Burgess (1959) found the response to warm moist air to fluctuate in cycles of 3—4 days. If the observer was isolated from the mosquitoes by air-tight plastic, the response to warm moist air was abolished, but it could be restored by administering a little carbon dioxide. Cyclic activity had been found by Rahm (1957b) to be associated with a moist object and not with a warm dry object.

Using the olfactometer technique of Laarman (1958), from which it had been concluded by van Thiel and Laarman (1954) that *Anopheles atroparvus* was attracted by body odour as well as by CO_2, Brouwer (1958) studied the responses of *An. stephensi.* He found consistent differences in attractiveness between individual humans that were independent of moisture, warmth and CO_2, and which he concluded to be due to sweat or body odour. Schaerffenberg and Kupka (1959), following up their original discovery that a distillate from blood was attractive to *Culex pipiens,* reported that a mixture of 4 amino-acids (actic 1 acid), ammonia and 3 methylamines was attractive not only to *C. pipiens* but also to *An. maculipennis,* although none of these compounds were attractive when tested singly.

Our own recent work, performed in collaboration with Mr. A. G. Carmichael, has searched for individual substances in blood that would prove attractive to *Ae. aegypti*. Our starting-point was the report made 40 years ago by Rudolfs (1922) that he found peptone and 4 amino-acids to attract *Ae. sollicitans*. Accordingly we obtained a number of protein hydrolysates that were available commercially either as bait attractants for fruit flies or as media for microorganisms. They were tested in 10 per cent solution added to filter-paper in petri-dishes warmed to 37°C and exposed in a cage containing adult females maintained only on sugar-water; the number of approaches as compared to those of a similar exposure of distilled water alongside was taken as the attractiveness ratio. Of 16 different protein hydrolysates tested, 11 proved to be highly attractive, and 8 of them were more attractive than beef blood plasma. The attractive principles proved to be distillable at reduced pressure in the case of 5 of these hydrolysates (Table 1).

Table 1

Attractiveness to female *Aedes aegypti* of protein hydrolysates before and after distillation, as compared with blood plasma

	pH	Attractiveness Ratio		
		Original	Distillate	Residue
Peptonized Milk Hydrolysate[1]	6.2	3.44	1.82	2.09
Casein Hydrolysate, enzymatic[2]	6.6	2.72	2.27	3.54
Yeast Hydrolysate, enzymatic[2]	5.2	1.83	0.23	2.99
Protein digest proteoses (Bacto-Protone)[1]	7.5	1.82	1.71	1.51
Casein digest, pancreatic (Bacto-casitone)[1]	7.1	1.80	0.98	2.47
Soy Hydrolysate, enzymatic[2]	7.3	1.72	2.36	1.57
Lactalbumin Hydrolysate, enzymatic[2]	6.6	1.51	3.00	2.14
Blood Plasma, Beef	7.4	1.48	1.68	1.57

[1] Difco.
[2] Nutritional Biochemicals Corp.

We then concentrated our attention on lactalbumin hydrolysate, using first the synthetic method and then the analytical method in trying to find the substances in it that were attractive. First the individual amino-acids were tested in the concentrations in which they are known to occur in 10 per cent lactalbumin (Table 2), using the natural L-isomers. Many of the amino-acids appeared to be actually repellent, only arginine and alanine showing a moderate attractiveness, but lysine was strongly attractive.

Table 2

Attractiveness ratios for natural L-isomers of amino-acids in the relation concentration occuring in lactalbumin

Lysine, 0.88%	3.84	Valine, 0.33%	0.85
Alanine, 0.24%	1.32	Glycine, 0.04%	0.81
Arginine, 0.30%	1.24	Histidine, 0.21%	0.75
Tryptophane, 0.27%	1.05	Isoleucine, 0.70%	0.75
Phenylalanine, 0.13%	0.98	Aspartic acid, 0.82%	0.70
Glutamic acid, 1.3%	0.96	Proline, 0.38%	0.69
Cystine, 0.11%	0.95	Leucine, 0.70%	0.68
Serine, 0.18%	0.89	Tyrosine, 0.05%	0.58
Methionine, 0.88%	0.88		

Lysine was not one of the amino-acids involved in the reports of Rudolfs (1922) and of Schaerffenberg and Kupka (1959).

A mixture of all the 16 amino-acids except lysine (p_H 3.4) was weakly attractive (Table 3); addition of lysine made the mixture (p_H 4.4) highly attractive. The results were similar when the mixture with or without lysine was tested at p_H 7.4, the p_H of human blood. Lysine is a strongly basic amino-acid, dissociating in water to give a p_H of 9.5; with a pK_3 of 10.5, it is thus not completely undissociated until the p_H is alkaline to 11. Lysine hydrochloride (Table 4) is unattractive in acid solution but its attractiveness increases as conditions are made more alkaline. Lysine free base is attractive even in acid, but is much more attractive in alkaline solution.

Table 3

Attractiveness ratios for the amino-acids of lactalbumin with or without L-lysine

16 Amino-acids without Lysine; p_H 3.4	1.14
16 Amino-acids plus Lysine; p_H 4.4	2.80
16 Amino-acids without Lysine; p_H 7.4	1.12
16 Amino-acids plus Lysine; p_H 7.4	6.13

Table 4

Attractiveness to female *Aedes aegypti* of L-lysine and its hydrochloride at various p_H levels. Control solutions identical except for absence of amino-acids

L-Lysine Free Base, 0.70 per cent		L-Lysine Mono-HCL, 0.88 per cent	
p_H	Attractiveness Ratio	p_H	Attractiveness Ratio
4.40[1]	5.62	5.52[1]	0.64
7.45[1]	3.23	5.95[2]	1.12
7.45[3]	3.03	6.73[4]	1.30
9.52[2]	3.84	7.38[4]	2.59
9.73[5]	5.09	11.20	3.33

[1] Phthalate-phosphate buffer.
[2] No buffer.
[3] Citrate-acetate buffer.
[4] Tetraborate-phosphate buffer.
[5] Tetraborate-carbonate buffer.

At p_H 10, lysine was highly attractive even when diluted to 0.0001 per cent (1 p. p. m.), 50 times more dilute than the threshold of the attractant mixture for *C. pipiens* (Schaerffenberg and Kupka, 1959). A distillate from 0.7 per cent lysine was also highly attractive, the attractiveness ratio being as high as 7.7. The unnatural isomer D-lysine, obtained as the hydrochloride, resembled L-lysine hydrochloride in being slightly attractive in neutral and highly attractive in alkaline solution.

In the analytical study, 10 per cent lactalbumin hydrolysate was chromatographed by the method of Moore and Stein (1954a) through a 50-cm column of styrene-divinylbenzene resin (Dowex 50 W). Successive samples of the eluate were tested for attractiveness after being made alkaline to p_H 10 (Fig. 1). The bulk of the attractive material was present in the samples between 70 and 90 ml. after the addition of the material to the column. With 0.1 per cent lactalbumin hydrolysate the peak was between 80 and 90 ml. There was another peak of attractiveness at 30—40 ml. with a slight peak at 110—120 ml.

The results obtained from chromatography of a mixed solution of alanine and lysine (Fig. 2) indicate that the main peak was due to lysine and the early peak to alanine; possibly the slight peak at the end is due to arginine. The eluate at 86 ml. from the less concentrated hydrolysate was identified as lysine by paper chromatography. Since traces of ammonia would elute close to lysine, solutions of ammonia were tested but were found unattractive in dilutions between 0.2 and 0.0005 per cent. Ornithine elutes even closer to lysine, and asparagine elutes close to alanine; but both when tested as L-isomers were found to be unattractive, even in alkaline solution.

Samples of fresh beef blood plasma, after removal of proteins with picric acid, were concentrated by evaporation and chromatographed through the resin column (Fig. 3). The eluates of both samples showed the main peak of attractiveness at approximately 90 ml. with a smaller peak at 40—50 ml. and slight attractiveness between 101 and 130 ml. Samples of human blood were then obtained from a blood bank, treated and chromatographed in the same manner; the eluates of both samples showed peaks at 40—50 ml., around 80 ml., and at 110—120 ml. The eluates at 44 and 81 ml. from the more concentrated blood were identified as alanine and lysine respectively by paper chromatography.

The results with lactalbumin hydrolysate and beef blood indicate that the most attractive principle is lysine. With human blood, the less concentrated sample showed the highest attractiveness in the neighbourhood of lysine, but the more concentrated sample showed it in the neighbourhood of alanine. At a buffered p_H of 7.4 alanine was more attractive than at the p_H of 6.8 obtaining when it is dissolved in water, and when tested against lysine at p_H 7.4 was almost as attractive as it. Then again when chromatographed and made alkaline to p_H 10 alanine is still more attractive, although not as attractive as lysine at this p_H. It is probable that when admixed with the other amino-acids the attractiveness of alanine is largely masked by their net repellency, but that it can express itself when separated by chromatography. Alanine was one of the amino-acids involved in the reports of Rudolfs (1922) and of Schaerffenberg and Kupka (1959).

Lysine, when its solution is exposed in a cage, incites the resting mosquitoes to take flight, approach and repeatedly sample the petri-dish, occasionally landing on the treated filter-paper; these responses closely resemble those made to a guinea-pig or a human hand introduced into the cage. Lysine was equally as attractive when the observer was isolated in air-tight plastic. Alanine also elicits these responses, but not so strongly. Consistent results are not abtained with lysine in an olfactometer of the type described by Willis (1947); indeed, consistent results could not be obtained with blood plasma in this apparatus, which after being used for 6 months of inconclusive results was abandoned for the free-flight exposure cage of Burgess and Brown (1957). In this latter apparatus lysine was highly attractive not only to the test strain of *Aedes aegypti*, which was founded from a single female of the well-known Orlando laboratory colony, but also to strains from Key West, Trinidad and Penang, even when they had been given daily access to a guinea-pig. Lysine also proved attractive to wild-caught *Aedes stimulans*, and to a laboratory strain of *Culex pipiens* when tested in diminishing light at evening.

The relation of these findings to the living animal remains to be seen. It is true that lysine and alanine are two of the three most abundant amino-acids in human blood (Moore and Stein, 1954 b). But it has yet to be demonstrated that they volatilize from the capillaries of blood or lymph. The present work does indicate that a balance between the attractants lysine, alanine and possibly arginine, and the more repellent of the other amino-acids, may have a relation to the host preferences of *Ae. aegypti*. For example, Rahm (1958) has evidence that it is the olfactory substances emitted from the skin which makes men consistently more attractive than women to this species. Smith (1956) has reported that a boy is more attractive than a man, per unit of surface, to *An. gambiae*;

although with *An. maculipennis* Reuter (1936) had found that amino-acids were not attractive. Dow, Reeves and Bellamy (1957) established that the relative attractiveness of different species of birds to *Culex tarsalis* was correlated with their size, which in turn determines their CO_2 output. Previous work has made it clear that attractiveness is due to a number of factors — warmth, moisture, CO_2, body odour and visual stimuli — and that their relative importance varies according to the situation and the species of mosquito. Once attracted, the stimulus to engorge on blood is evidently due to another factor, identified as adenylic acid by Hosoi (1959) for *C. pipiens pallens*. But it is possible that the differing preferences of various species, and even of anthropophilic and zoophilic strains of the same species, may be mainly attributable to their responses to particular amino-acids in the body emanations, of which the most important is lysine.

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THE ATTRACTION OF FEMALE MOSQUITOS (Aedes aegypti L.) TO OLFACTORY STIMULI

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The reaction of equally raised and starving *Aedes aegypti* females of the same age to various human body fluids was tested in an olfactometer to search for the attractive compounds of blood to mosquitos. We preferred for the investigations an olfactometer similiar to that used by Willis in order to work with constant temperature, moisture and light conditions. Filterpaper soaked with the substances were warmed in vaporising cylinders in the olfactometer. In other experiments an airstream was bubbeld through a solution of test materials. In both tests only airborne factors could reach the animals. The test worked as a choice-test for 50 females between two equivalent air currents, one loaded with the odours. The percentage difference between the mosquitos alighting within the same time on the emission ports was taken as indication of olfactory attraction.

We received the same results with beefblood as Burges & Brown did, but we found also a pronounced attraction by urine. After hydrolysis and extraction of the sample with ether one of the fractions obtained proved highly attractive even after great dilution. This fraction contained diphenols such as resorcine, hydroquinone, and catechol, all

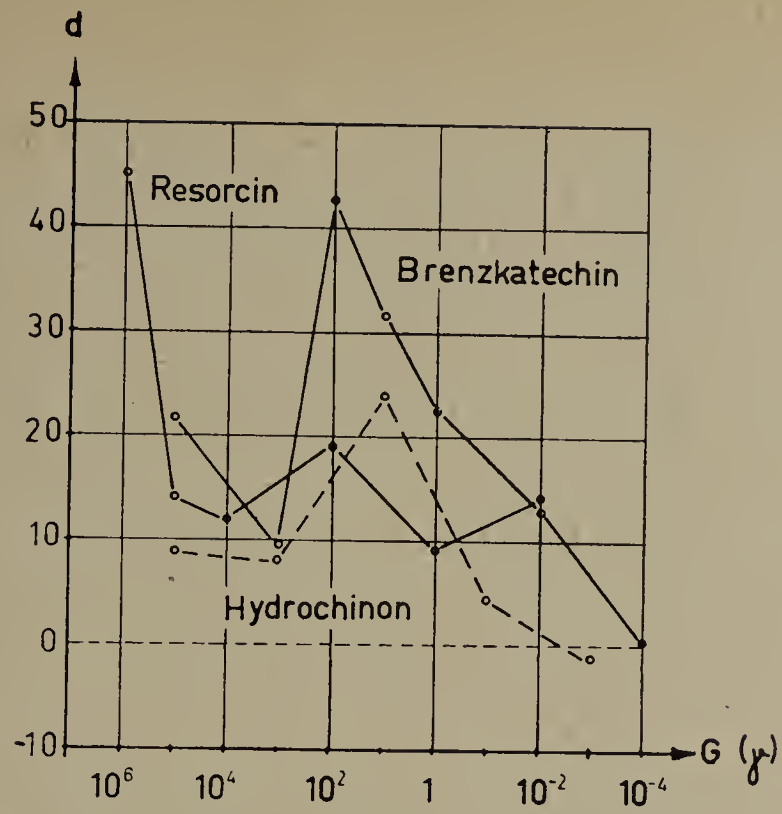

Fig. 1. Correlation of the average difference (d) of percentage of alighting females on the emission port to the amount of diphenols brought to evaporation.

of which showed statistically significant stimulation, though phenol itself was a strong repellent (Fig. 1). Paperchromatograms of this fraction were cut into strips as indicated by the position of certain control substances from simultaneous runns. After evaporation of the solvents the strips were offered to the mosquitos in the olfactometer. The paper-strip (0.67—0.9 Rf) loaded with estrogens gave the highest effect (Fig. 2). Some other highly attractive spots could not yet be identified. The mosquitos were sensitive to some purified estrogens, which were kindly given us by Prof. Butenandt, Muenchen.

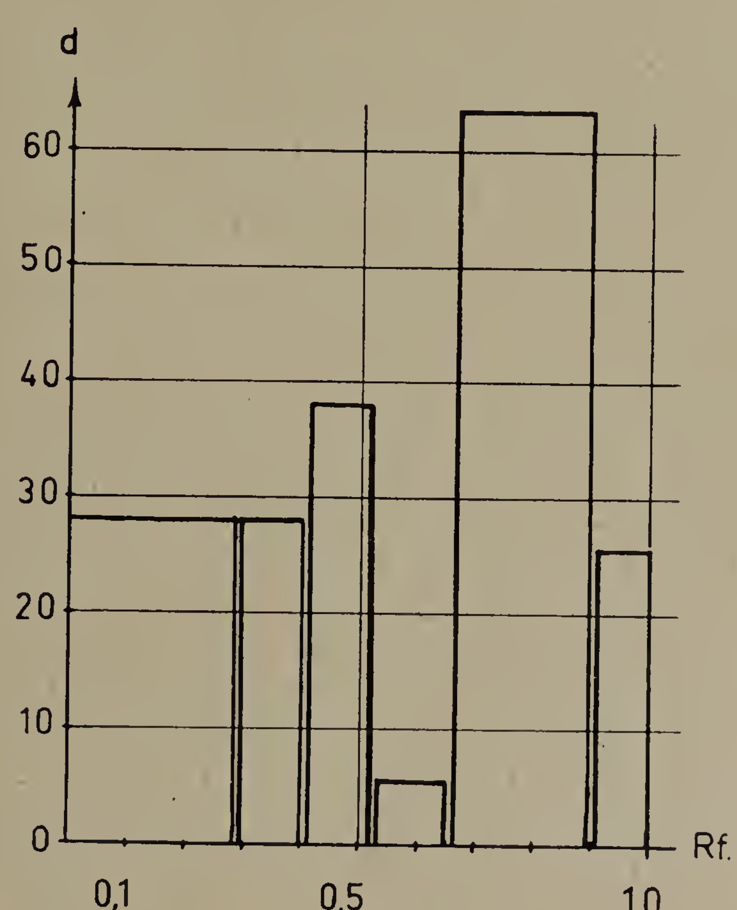

Fig. 2. Difference (d) of alighting mosquitos on the ports correlating to vapors originating from some strips of paperchromatogram of the phenolic urine fraction.

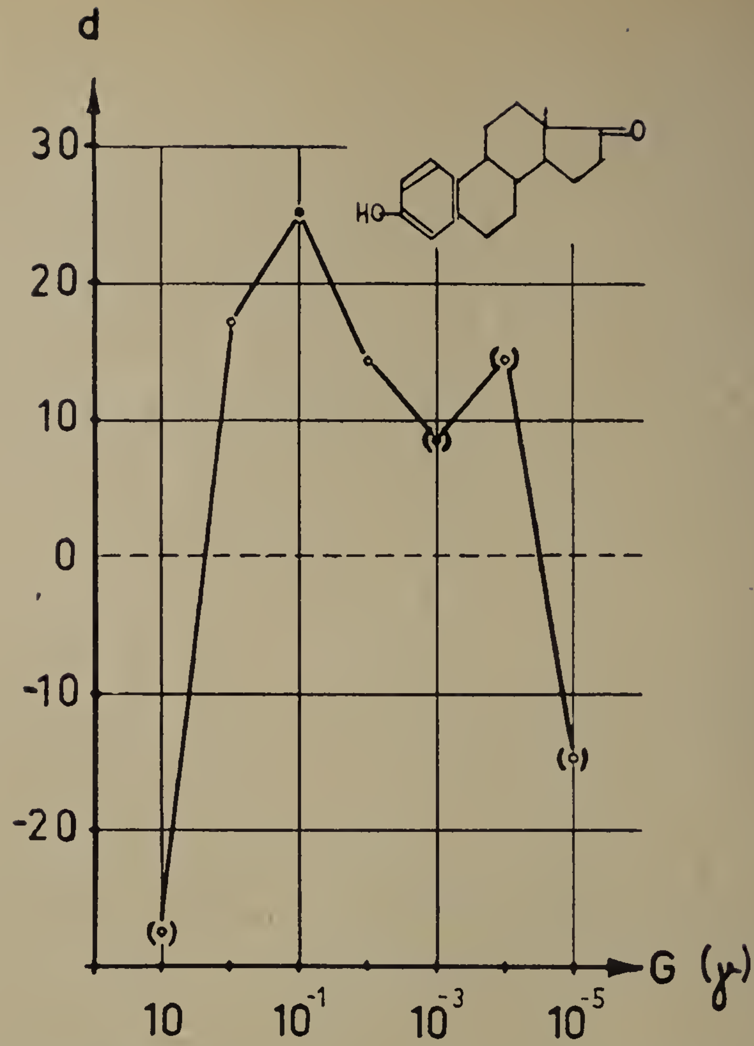

Fig. 3. Correlation of average difference (d) of percentage of alighting females on the emission port to the amount of *estron* brought to evaporation.

The response of the mosquitos to increasing dilutions of estron and estriol may suffice as an example for steroids with similiar effects. Each dot is an average of 10 to 20 repetitions done with groups of 50 females. The mosquitos reacted to as little as 10^{-9} micrograms of estrogen evaporated from a solution on filter paper. The animals reacted positively to 10^{-5} μg of equiline and, to a lesser degree, to an equal amount of androstandion 3,17. Remembering that only airborne molecules influenced the females and not the whole amount brought on the piece of paper, one can imagine the specific sensitivity of mosquitos to estrogens (Fig. 3 and 4).

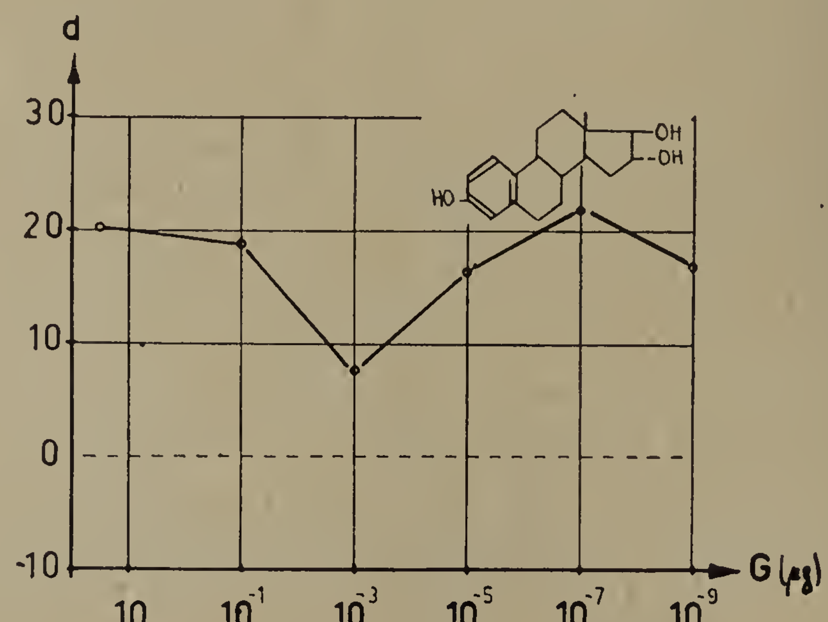

Fig. 4. Correlation of the average difference (d) of percentage of alighting females on the emission port to the amount of *estriol* brought to evaporation.

The inquiries made by Schaerffenberg & Kupka on the attraction by amino acids were repeated, using the described olfactometer. It could be shown, that methionine, a mixture of glycine, alanine, valine, leucine or lysine and arginine had no significant effect on the mosquitos. On the other hand, as might be expected, the phenolic amino acid tyrisone showed a significant attraction. In the same way combination of asparatic and glutamic acids, serine and threonine, and also proline and histidine stimulated the females (Table 1). I do not want to controdict Prof. Brown's results and his excellent work just mentioned. I am sure, that the few differences are only the results of the effect of different concentrations or of our differing experimental techniques. Further investigations will bring some light to these questions. A comparative test of urine fractions showed that whether male-urine or pregnancy urine was used, the phenolic fraction was in both cases significantly more attractive then the water-soluble fraction, which contained only amino acids. The attractive body odour seemed to consist of several compounds supplying and substituting each other, but did not act only as a complex. The phenolic substances, especially the steroids, were found to be the most effective and specific ones.

It is suggested that differences of attractiveness of the individuals to the mosquitos depends on the varying amounts of steroids in the body fluids. Through short distances the female mosquito is guided to its host by a humidity and temperature gradient,

Table 1

Amount in µg of substance brought to evaporation			n	Alights	st
Methionin	22		8	48.2	0.45
		$\cdot 10^{-2}$	11	53.8	0.69
Glykokoll Alanin Valin Leucin	30.5 30.0 24.0 22.5		20	53.0	0.96
		$\cdot 10^{-2}$	16	54.6	2.15
Lysin Arginin	26.2 200.0		12	56.1	0.90
		$\cdot 10^{-2}$	10	63.3	2.65
Tyrosin	24.0		20	59.8	3.1
		$\cdot 10^{-2}$	10	63.6	3.25
Asparaginsäure Glutaminsäure	87 99		20	52.0	0.96
		$\cdot 10^{-2}$	12	70.6	4.32
Serin Threonin	450 32.9		12	68.0	5.68
		$\cdot 10^{-2}$	10	69.7	7.26
Prolin Histidin	27.5 19.8		12	65.5	4.88
		$\cdot 10^{-2}$	12	68.5	6.06

n: Number of experiments with groups of 50 females.
Alights: Percentage of alighting females in average on the attraction port.
st: student's error.

through the ascending CO_2-concentration indepentent of the air current and by visual factors. Over long distances the mosquito will only be stimulated by the odours of the host and perhaps by CO_2 to fly characteristically windwards to its prey.

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DISCUSSION

JONES: 1) What did the mosquitoes do in the presence of $10^{-9}\gamma$ estrone or estriol?
2) Did you dilute these steroids further than 10^{-9}?
How did you determine the number of molecules present in the air at the port?

ROESSLER: 1. Die weiblichen Stechmücken versuchen in den Anlockexperimenten mit Oestrogenen durch das Gazegitter zu stechen und zeigen typische Suchbewegungen wie auf einem dichten Kleidungsstück, während sie auf der Kontrollöffnung ruhig sitzen bleiben.

2. Die Anlockung wurde bisher nur bis zu Verdünnungen von $10^{-9}\gamma$ Oestrog untersucht. Für weitere Verdünnungsstufen muß eine besondere Versuchseinrichtung entwickelt werden. Aus dem Molekulargewicht und der Loschmidtschen Zahl wurde eine mutmaßliche Molekülkonzentration in Luft berechnet, vorausgesetzt, daß die gesamte zur Verdampfung aufgetragene Substanzmenge in die Gasphase übergeht. In diesem Fall würde für $10^{-9}\gamma$ Oestriol etwa 45 Moleküle durchschnittlich im cm^3 Luft enthalten sein. Eine solche Substanzmenge kann natürlich chemisch nicht mehr nachgewiesen werden.

JONES: Did you find that the mosquitoes fed on or probed the lysine in the same manner as a guinea pig? Do you find lysine in the sweat?

BROWN: The concentration of amino-acids in sweat is low. The attractiveness of sweat is also low as compared with that of body odour. It would appear that the volatilized amino-acids originate not from the glandular secretions of the sweat cells, but from the skin as a whole.

BAR-ZEEV: I wish to comment on the statement of Prof. Brown that mosquitoes are attracted to moisture. As a matter of a fact there is a discrepancy in the literature on the effect of moisture on mosquitoes. We found that mosquitoes strongly avoid a wet surface. However, if they lose a certain amount of water they become attracted to a moist surface. We believe that the reason for discrepancy is to be found in the water balance of the mosquitoes and in the techniques used, whether all of the mosquitoes take part in the reactions or only a small percentage. Your result that a sweat surface was less attractive is most probably due to the fact that non-dried mosquitoes avoid a wet surface. We found that mosquitoes did not bite a wet human skin.

BROWN: Perhaps we should say that our experiments finding moisture to be unattractive did not involve free moisture. It was simply that the hands of those people who had been found when at rest to transpire more moisture were later found to be less attractive than hands which had transpired less moisture. In fact, one man of the so-called Caucasian group who was outstandingly moist-handed proved to be outstandingly unattractive to *A. aegypti*, and on later enquiry proved to be of Hungarian (non-Caucasian) parentage.

CLEMENTS: A very wide range of organic compounds which are attractive to the housefly has become known. Could you summarize the compounds which you and Dr. Roessler found attractive? Could you say whether the substances are more likely to emanate from the skin rather than from the blood?

BROWN: Compared to the housefly, mosquitoes have a very narrow range of attractants; in fact it is only very recently that any mosquito attractants have been discovered. One might say that they fall into 2 groups—aminoacids and polycyclic phenols (steroid oestrogens). A full range of housefly attractants were tested 40 years ago by Rudolfs on Aedes mosquitoes, and he found only peptone, 4 amino-acids, haemoglobin (sic!) and a benzoic acid derivative to be attractive to the mosquitoes. As to whether the attractant in an animal's body originates from the skin or from the blood, one would say that it is emitted from the skin but that it must have been transported there in the blood and lymph. That the greater attractiveness of vasodilated subjects is due to increased volatilization from the blood remains to be yet demonstrated.

LAARMAN: Ribbands found that although one person may attract considerably more mosquitoes than others, simultaneously tested, for a number of consecutive days, he may suddenly

cease to be the most attractive. I think that these recent discoveries of amino acids and steroids, neither of which is likely to occur at a constant level in one individual, as attractants, offer a good explanation for this phenomenon.

BROWN: Perhaps one should say that any conflict that may appear between our results originates from the fact that it is not until now that we have been aware of each other's findings. Obviously they derive from the 2 different test methods used, and the different focus of interest on chemical groups. Perhaps it should be pointed out that lysine has an attractiveness ratio of about 8 times while that obtained in the olfactometer experiments with other amino acids and steroids was only about 2-fold.

We should examine the purpose of such studies, and it is twofold. Probably the most important is to ascertain what material in the body odour of man, mammal or other vertebrate makes it attractive in the normal physiological condition. But subsequently one can probe the range of possible attractant compounds, the most attractive of which may appear to be entirely non-physiological or quite unknown in the body. It is clear that both of us must return to the laboratory to reconcile the conflicts in our results, e.g. particularly with tyrosine, which may be due to concentration reversal, so that by the time of the next Congress it will be possible to present a sensible picture.

THE INFLUENCE OF ILLUMINATION UPON THE DIRECTION OF THE ESCAPE FLIGHTS OF ANOPHELINES IRRITATED BY DDT

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México, D. F.

Kennedy was presumably the first who observed in the laboratory that mosquitoes get irritated by contact with DDT-covered surfaces, and fly off; in 1947 he published a detailed paper on the irritability of *Anopheles maculipennis atroparvus* and *Aëdes aegypti.* Experiences in the field (Gahan, 1945; Muirhead-Thomson, 1951; Reid and Wharton, 1956; Trapido, 1952, 1958), especially in experimental huts provided with exit traps, showed that under certain circumstances the irritated mosquitoes escape into the open air.

Therefore the irritation of mosquitoes at contact with DDT became, during the last few years, the object of several investigations by Coluzzi (1958), Brown (1958), de Zulueta (1959) and by our study group in Mexico (Barrera; Hecht; Hernández-Corzo; Mancera; 1958, 1959 and 1960). In our laboratory tests with *Anopheles* (*N.*) *albimanus*, *A.* (*A.*) *quadrimaculatus* and *A.* (*A.*) *aztecus* we found that great differences respecting irritability exist among the various species and that among the two first mentioned species the unfed females are much more irritable than those who had obtained a bloodmeal about twelve hours earlier.

After having dealt with the quantitative study of the mere irritability of mosquitoes by contact with DDT, we took up the question to which escape reactions the irritated mosquitoes can be induced, i.e. we wondered which environmental factors may direct the flights of the mosquitoes into certain directions, perhaps the differences of light, temperature and humidity existing between the interior of a sprayed house and the open air, as well as air currents, the presence or absence of animals, human beings, etc.

One circumstance which can influence the direction of flights is the difference in illumination between the various parts of a house or a room, or between the inside and the open air.

In this short report I must restrict myself to describing only our most important experiments, the observations we made in a spacious device, as tests in smaller containers could, on account of the close confinement of the mosquitoes, produce somewhat varying results and as they would be performed under circumstances rather different from the natural conditions prevailing for example in a sprayed hut.

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Our contrivance for these experiments consisted of a number of square wooden cases of ½ m side length, the interior of which was covered with paper treated with a DDT aceton solution, so as to obtain a cover of DDT crystals corresponding to 0.5 g/m² of active substance. The top of the boxes consisted of a transparent plastic sheet (crystal acetate), a material to which anophelines can not cling. By covering this sheet with some fabric or black paper, the interior of the boxes could be darkened in various degrees. In the middle of one wall, or in some cases on two opposite sides of the boxes were quadrangular openings, 20 × 20 cm, to which tunnels of 1.50 m length could be attached. The ceiling of the tunnels was a transparent sheet, the sides consisted partly of mosquito gauze. Into the quadrangular openings of the boxes open truncate four-sided pyramids made of crystal acetate were inserted which jutted into the tunnel with their narrow part, i. e. a quadrangular opening of 4 × 4 cm. These funnelshaped structures did not hinder at all the flight of the mosquitoes from the boxes into the tunnel, however they made a return flight into the boxes quite difficult.

In each single experiment 50 mosquitoes were introduced into the box. The mosquitoes which had flown out into the tunnel were counted every 15 minutes at the beginning of the tests, later on only every 30 minutes; and the observations were continued up to 3—4 hours.

In a first series of tests we allowed daylight to enter unhindered into a box and darkened the tunnel. As a parallel to this arrangement another box was slightly darkened by partly covering its skylight, whereas the tunnel remained entirely illuminated. The observations of the first arrangement showed that the irritated females, hungry as well as bloodfed ones, of *A. aztecus* and *A. quadrimaculatus* flew without exception into the dark within a very short period. When in opposition hereto, the mosquitoes of the second arrangement had only the opportunity to fly towards light, the hungry *A. aztecus* showed a slight tendency to do this, bloodfed *A. aztecus* and unfed *A. quadrimaculatus* an even lesser inclination, and bloodfed *A. quadrimaculatus* did not fly towards light at all. Of the irritated unfed and bloodfed *A. albimanus* about 75% escaped into the dark, when this opportunity was offered without choice; if, however, the only possible flight direction was towards light, then about 80% of the hungry and only 30% of the bloodfed females took same.

When in another series of tests which was executed in a much but not entirely darkened room, the differences of light between the box and the tunnel were only slight, then about 50% of *A. aztecus* flew into the only slightly lighter tunnel and—only 10% to 20% of *A. quadrimaculatus*. Under these circumstances only a small number of *A. albimanus* were flying into the tunnel; about 40% of the unfed and hardly any of the bloodfed females. In an analogous manner to these tests made in artificially darkened rooms in daytime, we observed the same behaviour of the different mosquitoes in tests at night. The results in both cases showed that a very moderate relative "brightness" in the tunnel which was really only a little lesser darkness than the obscurity in the DDT box, to a certain degree already counteracts the escape of the irritated mosquitoes.

In our night tests we made some in which the interior of the boxes was illuminated brightly by fluorescent light. This light drove away completely and very quickly the *A. aztecus*; *A. quadrimaculatus* in a high percentage but more slowly; and of *A. albimanus* approximately 50% within one hour.

In a last series of tests *two* tunnels were attached to a DDT box on opposite sides, a darkened and a lightened one. *A. aztecus* and *A. quadrimaculatus* escaped in full numbers into the dark tunnel within a particularly short time. Of the unfed females of *A. albimanus* 58% flew into the dark, 42% into the light tunnel. Of the bloodfed females of *A. albimanus* 56% flew into the dark, 24% into the light tunnel and the remaining 20% stayed in the box.

The tendency of the irritated mosquitoes to fly towards the dark is slightly intimated in the control experiments when mosquitoes were introduced into the large boxes *without* DDT, if those mosquitoes not irritated by DDT flew off at all.

On the basis of all these mentioned tests we realized that *A. aztecus* and *A. quadrimaculatus*, in case they should find their way out of a sprayed house, had not been attracted by light. The irritated females of *A. albimanus*, a semi-exophilic species, show a little different behaviour; though they also escape to a great part towards darkness, they do not refrain from flying towards lighter places too.

In spite of our findings, the validity of the observations made by different authors concerning the escape of irritated mosquitoes from sprayed houses or huts is by no means being questioned. But, at least as far as the species we tested are concerned, under natural conditions it is not the difference of brightness existing between the comparatively darker interior of a house and the open air which direct the flights of irritated mosquitoes towards the open. There must be other factors which determine the flight of the irritated mosquitoes into the open.

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DISCUSSION

BARNETT: It was pointed out by me in a short note in Mosquito News (1954) that cellulose acetate sheeting is commonly treated with dimethyl phthalate as a plasticizing agent, and that such material, when used in the preparation of cages for experimental work with mosquitoes, could greatly affect the results of the experiments.

Do you have any information on the source of your material or its preparation?

HECHT: I have no information about the fabrication of this material. But we did never observe a detrimental or disturbing effect of cellulose acetate sheets, of which only limited parts of the apparatus were built. I compared our apparatus with a house and the results were completely in accordance.

FACTORS IN THE ATTRACTION OF MOSQUITOES TO HOSTS, AND THEIR RELATION TO PROTECTION WITH REPELLENTS

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Carbon dioxide, moisture, heat, color, and movement have all been shown to function in the attraction of mosquitoes to hosts, to different degrees and with different interactions depending on the test conditions. It is not our purpose to review the many contributions in this field, as a paper given at the Tenth Congress by Brown (1956) covered them ably. We will present the results of experiments on the relative attractiveness of different men and women and the relationship of this quality to the protection obtained with repellents. The results of a few tests on the action of adenylic acid will also be given, and studies on the responses of mosquitoes to a man enclosed in a light-weight rubber diving suit will be discussed.

The tests on the relative attractiveness of different individuals and the protection obtained with repellents were all conducted with *Aedes aegypti* (L.). The methods used to determine the biting rates on untreated arms, and the protection time with repellents are given by Smith (1960).

In studies of the relationship between individual attractiveness and the protection obtained with repellents, a series of tests was first made to determine individual differences in the protection period with dimethyl phthalate on six male subjects, and the amounts remaining on their arms at the time mosquitoes began to bite. The repellent was applied at about 11 mg. per 10 square centimeters, and the amount still remaining at the time 5 bites were received in a 3-minute exposure period was determined. The average amount of repellent remaining on the arms at the time of biting (6 tests) was very uniform on five of the subjects, ranging from 6.35 to 6.87 mg./10 sq. cm., although the protection times ranged from 99 minutes on Subject C to 200 minutes on Subject A. It was 172 minutes on Subject B, who, with A and C, took part in many later experiments. Protection times on these subjects were inversely correlated with the rate of loss, which ranged from 0.031 mg./10 sq. cm. per minute on Subject C to 0.015 on Subject A. It was 0.020 mg. on Subject B. However, Subject E was an exception, as he received bites after only 52 minutes while 8.80 mg. was still on the skin, a loss of 0.023 mg./minute. A second, more extensive series of tests with Subjects A and C gave similar results.

The relative attractiveness of Subjects A, B, and C was determined in biting rate tests; A received significantly more bites than C when no repellent was used, and B received 3 times as many. There was therefore no relationship between natural attractiveness and failure of the repellent in the previous two series of tests, since Subject C, who was the least attractive, received the shortest period of protection, and B, who was the most attractive, had protection periods about equal to A.

Additional experiments by Gouck and Bowman (1959) demonstrated that the arms of B gave off the most carbon dioxide and the least water, those of C the least carbon dioxide and the most water, and those of A intermediate quantities. The application of repellents to the arms did not affect the release of moisture, but dimethyl phthalate, ethyl hexanediol, and deet reduced the carbon dioxide output of A and B, and deet (N,N-diethyl-m-toluamide) reduced it on C also.

In other tests with three repellents on three men and two women (Smith 1960) there was no correlation between the relative attractiveness of the subjects and the amount of protection received, and only incomplete correlation between the rate of loss and the protection time.

Ethanol solutions of repellents mixed with sweat from the arms, and dimethyl phthalate saturated with carbon dioxide, alone and with water, were as effective as solutions of the repellents alone, when applied at the same dosage levels.

Studies were made of the influence of hair on the relative attractiveness of Subjects A, B, and C, and on the protection obtained with repellents. The left arm of each subject was shaved; the amount of hair removed was 10.7 mg/10 sq. cm. from A, 5.6 from B, and 8.21 from C. Biting rates on the shaved arms were almost identical whereas those on the unshaved arms showed the characteristic differences.

Paired tests were made with dimethyl phthalate, deet, and ethyl hexanediol applied to one shaved and one unshaved arm of each of the three subjects. The rates of loss were generally equal or about equal on shaved and unshaved arms, but when differences did occur the faster loss was on the shaved arm. Differences between protection periods on shaved and unshaved arms were greater but less consistent, the longer periods sometimes occurring on the shaved arms and sometimes on the unshaved.

Tests were conducted to determine whether the sebum on the arms of B and C contributed to the attractiveness. The amount of sebum recovered from Subject B was 0.069 mg. per 10 sq. cm. of skin per day, and that from Subject C was 0.042 mg. Tests were made to compare the biting rates on the arms of the subjects when the sebum was rinsed from one arm by submersion in acetone and 1 ml. of acetone was spread on the other arm to serve as a check. The opposite arms of each subject were tested against each other and against each arm of the other subject. The rinsed arms of B and C received fewer bites than the check arms, but the difference, though marked, was not statistically significant at the 5% level. As in previous tests, B's check arm received significantly more bites than C's check arm, but when the arms of both subjects were rinsed part of the difference was lost (it was now significant at the 7% level) and there was no difference between the rinsed arm of B and the check arm of C.

Other tests were made to observe the effect of adding the extracted sebum to the rinsed arms. In this series rinsing the arms to remove the sebum did not decrease the number of bites received, and adding sebum did not increase the biting rate.

Hosoi (1959) reported that 5′-adenylic acid and related compounds are the materials in blood which induce feeding by mosquitoes, and found they induced feeding on saline solution at p_H 7.00. Confirmatory experiments were made with 5′-adenylic acid as a 0.0001 M solution in a saline medium at p_H 7.00 and as a 0.001 M solution in saline at p_H 7.00 and 3.65 and in distilled water at p_H 6.80 and 3.70. The saline solutions were 0.15 M. Since the p_H was adjusted with sodium hydroxide, some sodium salt of the adenylic acid was present in the saline at p_H 7.00 ana the distilled water at p_H 6.80. Saline and distilled water without adenylic acid were used as checks. All the liquids were warmed and offered to female *A. aegypti* in membrane-covered cylinders. From 68% to 81% of the mosquitoes fed on the 0.001 M solution at p_H 7.00, and 58% fed at p_H 3.65, whereas no more than 11% fed at 0.0001 M, no more than 2% on the saline check, and none on distilled water. The presence of sodium chloride, and probably heat, as well as the concentration of adenylic acid influenced the feeding.

Warmed and unwarmed saline solutions, with and without 0.001 M of 5′-adenylic acid, were compared. Both heat and acid were necessary to induce feeding. Mosquitoes fed about equally well on warmed saline solutions containing the acid at 0.001 and 0.002 M, with p_H's of 3.5, 5.0, and 7.0.

Tests were also conducted with glass traps made of drinking glasses with screen funnels which were exposed for 10 minutes in cages of about 800 mosquitoes. A 0.001 M concentration of adenylic acid in saline at p_H 7.00 caught about 50% more mosquitoes than saline alone (70 vs. 47, average of 6 tests) when the solutions were warmed to

45°C. at the start of the test, but less than the saline alone in two tests with unheated solutions.

Studies were made of the responses of mosquitoes to a man in a light-weight rubber diving suit. The diving suit completely covered the subject and prevented the release of water vapor, carbon dioxide and other gases into the environment. The exhaled air was carried to the outside of the building by a vacuum pump, except in tests with the face exposed. The test inclosure was an 8- by 8-foot room with double walls and two double observation windows, constructed within a larger room, and stocked with 300 female mosquitoes. The room contained water for humidity, honey solution for food, a carbon dioxide tank, and three dummies of roughly human size and shape, clothed in white cotton suits. It was illuminated by a 150-watt bulb and was maintained at an average temperature of 79°F. and 55% relative humidity.

After the experimenter had donned the diving suit, the outside of the suit was washed with hot soapy water and rinsed with cold water by a man wearing rubber gloves, to remove any human odor remaining on the suit from handling while dressing. Observations were made on the behavior of the mosquitoes while the experimenter was wearing the diving suit alone, and with a series of white cotton suits over the diving suit. Cotton suits were tested in the following order: dry, cool damp, warm damp, and worn. All except the worn suit were rinsed in acetone to remove human odor. The diving suit was then tested again, followed by tests with the hands and/or face exposed, with and without a white dry suit over the diving suit. Finally, a worn white suit was tested without the diving suit underneath. During the next week a similar procedure was followed, except that, after the first test with a diving suit, carbon dioxide was discharged from a pressure cylinder over the heads of the subject and one dummy at 0.5 liter per minute, about the rate it would be given off from the lungs and skin of a man. The subject remained in one spot in the test room while two men made counts of the mosquitoes on the subject and dummy through the observation windows.

Aedes aegypti were active flyers and landed on most surfaces in the room. The number of lands on the dummy were fairly consistent, and the average total count was 3.77 per half minute, which may be considered as the number landing on an object about the size, shape, and color of the experimental subject. When the subject wore the diving suit alone, the total number of lands was about the same as on the dummy when no carbon dioxide was released; with carbon dioxide the number was significantly higher than without, and also higher than on the dummy. When a dry white suit was worn over the diving suit, the number of lands without carbon dioxide was higher than on the diving suit alone, even with carbon dioxide, and carbon dioxide increased the landing rate still further. A cool damp suit was more attractive than a dry suit without carbon dioxide, less attractive with it. A warm damp suit was less attractive than a dry suit, both with and without carbon dioxide. A worn suit was less attractive than the clean dry suit without carbon dioxide, and equal with it. These differences, though statistically significant, were not great.

When the diving suit alone was worn, the total number of lands was higher with the hands exposed than unexposed, both with and without carbon dioxide, and was higher with carbon dioxide than without. Under these conditions, most of the mosquitoes landed on the hands and relatively few on the body without carbon dioxide; with carbon dioxide there were about equal numbers on the hands and body. When the face was exposed there was no increase in the landing rate over that with the complete diving suit, with or without carbon dioxide. When the face and hands were exposed together, however, the rate was significantly higher than with the hands exposed alone. Carbon dioxide increased the landing rate. The largest part of the count occurred on the hands.

When the hands were exposed, and a white suit was worn, the count was significantly higher than with unexposed hands when no carbon dioxide was released; with carbon dioxide the count was higher than without. The count was mostly on the hands without carbon dioxide, but mostly on the body with carbon dioxide. When the face was exposed the count was lower than when the mask was worn, both with and without carbon dioxide. When both hands and face were exposed counts were lower than when the hands alone were exposed, both with and without carbon dioxide, and more mosquitoes were on the body than on the hands and face.

Without the diving suit, counts were significantly higher than under any other condition (avg. 22.9), but less than twice as high as when the white suit was worn over the diving suit. Again, the additional carbon dioxide increased the landing rate. In both cases the majority were on the body, especially around the lower trouser legs and cuffs.

In summary, *aegypti* was found to respond readily to a man in a diving suit under a cloth suit. Exposing the hands increased the number of lands, whereas exposing the face either failed to increase or actually decreased the landing rate. The effect of a moist landing surface was variable and not very pronounced. Additional carbon dioxide usually increased the landing rate, but a reversal occurred with a cold damp surface.

Tests were also conducted with *Aedes taeniorhynchus*, the salt-marsh mosquito. Very few of the mosquitoes could be seen either flying or perched in the open. Lands on the dummy were uniformly low—below 1.5 per half minute—in all tests, and usually below 0.5 in the tests without carbon dioxide. In the series of tests without carbon dioxide, the only appreciable number of mosquito lands on the subject were in tests in which the face was exposed, the exact reverse of the results with *aegypti*.

With carbon dioxide present, lands increased noticeably in all tests with the exception of those in which the face was exposed. The number increased by a factor of 16 (0.41 to 6.41 lands) on the diving suit alone, 14 on the dry suit (0.50 to 7.24), 11 times on the worn suit (0.37 to 3.91), and 8 on the warm damp (0.75 to 6.45) and cool damp suits (0.67 to 5.46). In all tests in which the face was exposed, however, there were actually fewer lands with additional carbon dioxide than without it.

The largest total number of lands occurred on the subject in the diving suit without a white over-suit, with hands and face exposed, but without additional carbon dioxide. Most of these mosquitoes landed on the head. The next highest count occurred on a normal subject, without additional carbon dioxide. Most of these mosquitoes landed on the body.

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DISCUSSION

BAR-ZEEV: At what rate was the CO_2 released?

SMITH: 0.5 Liter of CO_2 discharged per minute, about the average amount given off by the skin and the lungs of a man.

BROWN: Might I ask Dr. Carroll Smith what method was employed for determination of CO_2 output from the arm; and whether Dr. Smith had considered eliminating the movement factor in his diver's-suit subject by use of a narcotic temporary paralysant, or anaesthetic?

GENERAL DISCUSSION

CORBET: I should like to comment on the distinction between the activity curve and the biting curve of mosquitoes, which I feel could have been more clearly stressed during the earlier remarks in which such phenomena were mentioned as: sporadic arrival at the host (the "pack effect"), the dilution effect proposed by Colless, and the possible build-up of biting intensity by progressive arrival of mosquitoes orientating towards the center of a study area.

We have recently obtained results in Entebbe which strongly support the idea that there is a well-marked cyclical *urge to bite* which is a far more important factor determining the pattern of the biting-cycle of some species than is the rate at which adults arrive at the host. In exactly comparable situations on a tower in forest in Uganda, we find that the non-specific flight activity of unfed females is cyclical, but that its pattern does not correspond to that of the biting-cycle. Thus females begin to arrive in numbers at a site between 01 and 03 hours, (as judged by light-trap catches), but they do not begin to bite there in appreciable numbers until 3 to 4 hours later. We have here a situation where females are arriving at a site, but not biting, even though a host is present. It would seem best to interpret this as being due to a cyclical fluctuation in the urge to bite. Somewhat similar observations have been made, I believe, by Love and Smith in America. Such considerations must seriously influence attempts to explain the pattern of biting activity mainly on the basis of random or orientated arrival, or on the basis of the depletion of mosquitoes which have previously arrived.

MATTINGLY: Work by Burgess, in Guelph, Ontario, recently published in Nature shows that a caged population of *Aedes aegypti* will periodically probe an ascending column of warm, moist air admitted through the floor of the cage. This probing is periodic and the rhythm involved is temperature independent and it may be that we are here confronted with a true biting rhythm as opposed to the composite entity which we term the "biting cycle".

Dr. Corbet's remarks regarding arrival during the middle of the night, i.e., in the case of *A. gambiae*, half way through the "biting cycle" could account for the imposition at this point of a convexity on the "biting curve".

ROESSLER: Ich möchte noch zwei Bemerkungen machen. Eine zu den sehr netten Experimenten, die Sie mit den Personen gemacht haben, die beieinander standen. Im Falle es tatsächlich die Steroide sind, die den größten Anteil an der Anlockung haben, ist es zu erwarten, daß die unteren Körperpartien stärker anlocken als die oberen.

Die andere ist: Es wurde berichtet, daß Büffel zu verschiedenen Zeiten verschieden starke Anlockraten haben für bestimmte Stechmücken. Das kann auf der Brunft dieser Tiere beruhen. Und in diesem Zusammenhang ist es auch wesentlich in den Versuchen, wo weibliche Personen und männliche Personen miteinander verglichen werden, acht zu geben auf den ständigen Wechsel an Steroiden bei den weiblichen Personen. Man kann auf diese Weise vielleicht erklären, daß an einigen Tagen weibliche Personen stärker Stechmücken anlocken als männliche. Es sind so viele Arbeiten über diese Fragen geschrieben worden, und es ist ständig ein Widerspruch gefunden worden. Die einen meinen, Frauen werden weniger angeflogen, die anderen erklären gerade das Gegenteil.

SYMPOSIUM VI

TRANSMISSION OF DISEASE BY TICKS AND OTHER ACARINA

THE POTENTIAL OF VIRUS-INFECTED AND TICK-INFESTED MIGRATING BIRDS IN THE SPREAD OF DISEASE

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Twice each year, millions of birds migrate intercontinentally from temperate to tropical climes and back again. The breeding and wintering areas, and migration routes and periods of some of these species are more or less well known. Less spectacularly, a large number of birds seasonally migrate shorter distances intracontinentally. Details and dynamics of these latter movements are infrequently if ever elucidated.

In the Western Hemisphere, at least 8 major flyways are recognized; these are almost entirely between areas within North and South America. In the Eastern Hemisphere, Africa receives birds from many parts of Europe and Asia. Individual species penetrate various distances into the continent, from Egypt to the Cape. Many fly directly from north to south, others veer to follow certain landmarks, as the Nile Valley or the Red Sea coast. Some Asiatic birds winter in East and South Africa after having passed over Arabia and/or the Indian Ocean from points east. In Asia, some routes pass at tremendous heights over the Himalayas, others skirt this massive range. Distant Ceylon is host to migrants from Russian Asia, the Kutch Peninsula of India is on the flyway of birds going either to southern Asia or to Africa.

Whether any of these migrants transport pathogens foreign to either of their seasonal habitats and the role of exotic pathogenic organisms in establishing extralimital foci of disease in man and animals are separate though related epidemiological questions of considerable interest. Potentially, even a single bird with circulating virus or reservoir ticks might serve to seed a new geographic area.

Definite evidence to prove the epidemiological role of migrating birds is difficult to obtain. To acquire such data demands a large scale, carefully integrated program with close cooperation between workers and specialists in virology, ornithology, and parasitology. This group must operate during specific weeks of the year, often in rather remote geographical areas away from established facilities. Highly specialized field and laboratory gear, equipment, and technical knowledge are required as well as considerable time. Another element, luck, always must be considered when searching for a needle in a haystack.

Today's theme, the potential of virus-infected and tick-infested migrating birds in the spread of disease, will be developed not so much to present evidence as to provoke thought that may lead to further efforts to elucidate this question. The subject matter is outlined on the following basis. Birds, often including migrants, are hosts of a number of viruses and rickettsiae pathogenic for man (especially causing encephalitis such as Eastern, Western, St. Louis, Japanese B, Murray Valley and several in the Russian spring-summer group; also Kyasanur Forest disease, West Nile fever, Q fever, etc.). Some of these are normally mosquito-transmitted, however adaptation to ticks apparently occurs in some instances. Ticks harbor and/or transmit an exceptionally large number and wide variety of pathogens, which they are capable of disseminating by one of several means. Certain viruses and rickettsiae survive for long periods in these arthropods. Developmental stages of numerous tick species parasitize birds and are not infrequently transported long distances by migrants.

A number of recent, isolated epidemiological incidents in the laboratory and field focus attention on the possibility of widespread virus dissemination through the agency of migrating birds and their attached ticks. This possibility was dramatized in 1957 by the sudden appearance in Mysore, India, of Kyasanur Forest disease, an entirely unknown, virulent, often fatal affliction of man and monkeys (Work, 1958). Tick-vectored, as are all viruses of the Russian springsummer group, of which this is a member, Kyasanur Forest virus or its neutralizing antibodies are found in forest monkeys, squirrels, insectivores and other small mammals. These animals share vector ticks, *Haemaphysalis spinigera*, with forest birds. It has been suggested that a migrating bird may have delivered this virus, either in its present form or in another which mutated in southern India, from far to the north where the R.S.S. virus group is endemic.

In Russia itself, a large volume of information gives us reason to believe that spring-summer encephalitis virus spreads through avian agencies. In reviewing the epidemiology of this disease, Pavlovsky (1940) and Smorodintsev (1958) indicate that birds are among the reservoirs of this virus and play an important role in transporting infected ticks, especially *Ixodes ricinus* and *I. persulcatus*, over great distances. Wild birds which are the most frequent carriers of ticks are the thrush, nutcracker bird, tree pipit, hammerbird, and others. Some of the birds mentioned here and elsewhere in relation to R.S.S. group viruses are the same ones that we find tick-infested as migrants in Egypt.

A second incident, reported by McIntosh (1959), is from the cold, dry, central highlands of South Africa. Here, active West Nile virus infection of man occurs under climatic and ecological circumstances that preclude continuous infection of endemic mosquitoes. Seasonal introduction of virus by migrating birds therefore appears to be a distinct possibility in this area. Similarly sporadic appearance of Eastern equine encephalitis in new foci in North America and of Murray Valley encephalitis in Australia also may be explained by movements of birds, as they are the principal vertebrate hosts of these agents.

A third group of findings suggests how ticks may be involved in cycles that normally alternate between mosquitoes and birds, with man tangentially becoming a host after having been bitten by infected mosquitoes. Taylor (1959) isolated West Nile virus from sick birds and from bird-infesting ticks, *Argas reflexus hermanni*, in Egyptian dovecotes during winter months when the normal mosquito-bird cycle of virus transmission is inactive. This discovery suggests not only that West Nile virus may adapt to ticks but also that ticks may serve as its overwintering reservoir. Earlier, Hurlbut (1956) had shown that *Argas persicus*, *Ornithodoros erraticus*, and *O. savignyi* become infected with West Nile virus during experimental feeding, and that *O. savignyi* may transmit the virus during feeding.

That *Argas* ticks may be transported by birds is indicated by the fact that we have found several larvae of this genus on migrants en route through Egypt from tropical Africa to Europe or Asia. Furthermore, the European bird parasite *A. r. reflexus* has been introduced into Egyptian bird nests where it appears to have interbred with the endemic *A. reflexus hermanni* (Hoogstraal and Kohls, 1960).

West Nile is not the only virus in the mosquito-borne category to be adaptable to ticks. Chumakov, Petrova, and Sondak (1945) reported that mosquito-borne, encephalitis-causing viruses from both Japan and maritime areas of Russia survived in *Rhipicephalus* and *Hyalomma* ticks and were transmitted through eggs to progeny and by bite to new hosts.

Although viremia in birds and other vertebrates may be transient and the infectious period only a matter of several days, many viruses survive in invertebrates during the entire life of the host. Thus, if a tick acquired virus from a migrating bird early in their association, the airborne tick and not the bird might carry the virus to a distant point.

At the final destination, virus dissemination by the tick might be accomplished by one of several means—by transmission during parasiting a new host in any successive developmental stage, by transovarial transmission to progeny, or when the tick is eaten by another bird or small mammal. In the case of *Rickettsia burnetii*, the causative organism of Q fever, viable pathogens may also be shed into the air with tick feces or upon the tick's death.

Another facet for consideration is geographic extension of known viruses far beyond classic foci, as shown in recent studies. Thus, as Rockefeller Foundation investigations extend deeper into South America, they reveal many unsuspected areas infected with encephalitis-causing viruses (Eastern, Western, and St. Louis) previously considered endemic in North America. These three, all bird adapted, may easily have been carried from north to south or vice versa by migrating birds. In this respect, the following remarks by Downs, Aitken, and Spence (1959) in reporting the first isolation of eastern equine encephalitis virus in mosquitoes in Trinidad, are of special interest: "It is noteworthy that the original *nigripalpus* strain was isolated (in Trinidad) in May, when bird migration was northward. The two *taeniopus* isolations (in Trinidad) occurred at a time when bird migrations had reversed and were headed southward."

A few words about a fourth American encephalitic agent, Venezuelan virus, might be interpolated here. This virus, confined to northern South America and forests of Brazil, and absent in North America, is not only more geographically restricted than the other three mentioned above, but its vertebrate hosts differ, being chiefly mammals and rarely birds. The inference here is that absence of infection in migrating birds may restrict the agencies for wide distribution of Venezuelan virus.

Another aspect to consider is that a multiplicity of new viruses are encountered whenever unstudied areas, vertebrates, or invertebrates are investigated. Results of the comparatively little research that has been undertaken on viruses of birds and ticks indicate how much basic research on this subject remains to be performed.

At NAMRU-3 in Cairo, study of endemic bird-infesting ticks revealed five unknown viruses, in two or more new groups, from *Argas persicus* and *A. reflexus hermanni* (Taylor and Hurlbut, 1958). One of these, Qaranfil virus, was also isolated from febrile children and sera from 4 to 16 percent of village residents possessed neutralizing antibody. The cattle heron, or buffbacked heron, *Bubulcus i. ibis*, apparently is an important host of Qaranfil virus. A number of other new viruses, including some from ticks and from birds, discovered recently in India, South America, and South Africa as a result of Rockefeller Foundation cooperative efforts, await further epidemiological elucidation. In general what results have been obtained have been from brief studies undertaken in addition to major investigative efforts.

Evidence that certain ticks do become temporarily or permanently established in new areas is provided from a number of reports. In some instances ticks from foreign sources are discovered on domestic animals under circumstances that can be best explained by avian introduction. Almost invariably, these tick species are those whose immature stages frequently parasitize birds. In other cases, the ticks are found on migrant birds and no question remains as to how they reached their destination.

Amblyomma variegatum, a species endemic in tropical Africa, has been recovered from dogs in France (Lamontellerie, 1954) and *A. hebraeum* of southern Africa was once taken from a cow in Bulgaria (Pavlov and Popov, 1951). *A. lepidum* of East Africa was found on the head of a stone curlew, *Burhinus oedicnemus*, in Azerbaijan (Pospelova-Shtrom and Abusalimov, 1957) and in Palestine has been taken sporadically from cattle as well as from a migrant European short-eared owl, *Asio f. flammeus* (Bodenheimer, 1937; Feldman-Muhsam, 1955). Another species of the Ethiopian Faunal Region, *Hyalomma marginatum rufipes* has not only become established in certain

areas of U. S. S. R. but, as noted below, is found in large numbers in Egypt on a variety of birds migrating from Africa to Europe and Asia.

Immature stages of the European-Asiatic *Hyalomma m. marginatum*, well known as a vector of pathogens of man and animals, especially of Crimean hemorrhagic fever, are frequently transported both northward and southward by migrating birds. In Africa, exotic populations of *H. m. marginatum* have been found in Sudan and in Kenya highlands (Hoogstraal, 1956) and, as discussed below, we find numerous larvae and nymphs in Egypt on birds en route to tropical Africa. Conversely, several Russian reports indicate temporary establishment of *H. m. marginatum* far north of its normal geographic range and its recovery from migrating birds.

Scattered reports from Europe and Asia refer to a variety of species in the genera *Hyalomma*, *Rhipicephalus*, and *Haemaphysalis* taken from migrating birds in Russia, Czechoslovakia, Germany, Sweden, and other countries; bird hosts mentioned are storks, nightingales, wagtails, owls, nightjars, pipits, and many others.

Between September and November 1959, we examined 8378 birds, representing 57 forms (species and subspecies), which had just flown over the Mediterranean from Europe and Asia and landed in Egypt en route to tropical Africa. Of these, 28 forms were carrying living immature ticks and 29 forms were totally uninfested. In the group of 28 infested forms, 7380 birds were examined; 320 of these bore 506 living ticks. Many other ticks were lost in handling; with better techniques the recovery rate would probably have been doubled.

The most productive hosts were the Willow Warbler, *Phylloscopus t. trochilus*, the Common Redstart, *Phoenicurus p. phoenicurus*, and the European Quail, *Coturnix c. coturnix*. Of the 5941 of these birds examined, 247 were infested, 306 ticks were recovered, and a large number were lost. Besides these three species, 25 other host species represented by 1440 birds were examined and 200 ticks were recovered from 73 hosts. These birds originated in Europe or western Asia and winter mostly in East Africa, some going as far south as the Zambesi River.

The following tick species and numbers were recovered from these birds: *Ixodes frontalis* (88), *Haemaphysalis* sp. (9), *H. punctata* (75), *H. sulcata* (2), *Hyalomma* sp. (236), *H. aegyptium* (11), and *H. m. marginatum* (66). Most of the *Hyalomma* sp. are probably *H. m. marginatum* a European-Asiatic tick that is represented among comparatively limited collections of adult ticks taken from domestic animals in Sudan and Kenya. It is not established in Egypt.

Studies of birds migrating northward through Egypt between 1955 and 1957 revealed 10 host species and parasitism chiefly by *Hyalomma marginatum rufipes*, an endemic tick of the Ethiopian Faunal Region which has become established in U. S. S. R. through the agency of migrating birds (Hoogstraal and Kaiser, 1958). Subsequent investigation has shown 22 species of birds flying to Europe and Asia to be infested by this tick when they reach the Cairo area.

These bits and pieces of epidemiological evidence, biological finding, and experimental results circumstantially suggest that the role of migrating birds and their attached ticks in the wide dissemination of pathogenic organisms may be more extensive than is generally appreciated.

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DISCUSSION

P. F. MATTINGLY: Further to Dr. Varmas remarks, I would suggest that, on the face of it, a tick represents a more favourable maintenance host for a virus than does a mosquito. This I believe to be the reason why the maintenance of virus in the northern steppes is dependent, as far as we know exclusively, on ticks. The curious thing is that in the tropics, where tick-born viruses exist, so many viruses should be carried by mosquitoes. It would, I think, be profitable to enquire under what circumstances ticks represent more favourable maintenance vectors than mosquitoes and vice versa. The findings could then be compared with the known relative distribution of these sorts of virus. Concerning the possible antigenic modification of viruses by introduction into new hosts or vectors it would be particularly interesting to know what happens to an African strain of yellow fever virus transmitted through a series of South American monkeys by *Haemogogus* mosquitoes.

H. BARNETT: In reply to Dr. Mattinglys suggestion that adaptation of viruses to new Arthropod species (non vectors) should be undertaken, it was stated that such studies are underway in our laboratories. We have found that after 4—5 passages of Japonese encephalitis virus in a species which is a poor vector, we obtained a virus strain whose transmissability was greatly modified, so that it could be readily transmitted by the poor vector species. However, at this passage level, no serological or immunological changes in the virus were detected.

ON NUTTALLIA ADLERI OF MERIONES TRISTRAMI[1]

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(See table VI)

A *Nuttallia* of *Meriones* was first found in Israel by Adler in 1930. This author reports that this *Nuttallia* is normally scanty and non-pathogenic; but after splenectomy of the host, it multiplies and may sometimes become fatal to the host.

[1] This investigation was supported in part by Reserach Grant R. G.-4531 from the National Institutes of Health, U. S. Public Health Service.

Identity

According to Capponi, Sureau and Deschiens (1955) only few species of piroplasms from rodents have been described until 1955.

Nuttallia decumani was found by Scott-Macfie (1915) in the brown rat (*Mus decumanus*). He did not succeed to transmit the infection to any of three white rats, by subcutaneous inoculations of contaminated blood.

Capponi, Sureau and Dechiens (1955) described a *Nuttallia* from *Rattus norvegicus*, in Viet-Nam. They succeeded to adapt it to the white rat and the white mouse. They think that this parasite might be the same as *Nuttallia decumani.*

Coles (1914) described *Nuttallia muris* from the field mouse and *Nuttallia microti* from the water vole (*Microtus amphibius*). As Coles does not report on any experimental work with the *Nuttalliae* he describes, it is very difficult to form an opinion on the identity of these species.

Two further species of *Nuttallia*, N. *golundae* Leger and Bédier, 1923, from *Golundae campanae* and *N. cricetuli* Springholtz-Schmitt, 1937, from *Cricetulus furunculus*, are mentioned by Capponi *et al.*; but as the original publications were, unfortunately, not available to us, we are unable to include these species in our discussion on the identity of the *Nuttallia* of the *Meriones.*

It is most probable that the *Nuttallia* of the *Meriones* is not identical with any of the known species of piroplasms of rodents, because none of them have been shown to be transmissible to *Meriones*. On the other hand, we did not succeed in transmitting the *Nuttallia* of the *Meriones* to the white mouse, the white rat, the cotton rat, the hamster, or the vole (*Microtus guentheri*). In splenectomized white mice, a transient infection lasting for about a day may be produced in very rare instances. However, in baby mice, Adler and Feldman-Muhsam (1952a) succeeded in infecting 100 per cent of the animals (73 cases) with blood of infected meriones; the *Nuttallia* multiplies in the blood of the baby mice, but after two days the infection diminishes gradually and after the ninth day, no parasites were found in blood smears.

It may be assumed that the *Nuttallia* of the *Meriones* is not identical with *N. muris* or with the *Nuttallia* described by Capponi *et al.*, for these piroplasms parasitize mice, which are refractory to the *Nuttallia* of the *Meriones.*

It is probably also not identical with *N. microti* as we failed to transmit it to any of ten baby *Microtus.*

N. decumani is also not transmissible to the white rat, but this is obviously not a sufficient reason to assume that *N. decumani* and the *Nuttallia* of the jird are identical. In fact, *N. decumáni* was originally found in *Rattus norvegicus*, and the *Nuttallia* of the jird seems to be highly specific to the *Meriones.*

In view of this specificity we are inclined to consider the *Nuttallia* of the *Meriones* as a distinct species. As yet no name has been proposed for this species by its discoverer.

Course of infection in mammal host

This piroplasm assumes various shapes inside erythrocytes, the most frequent one being that of a ring (Fig. 1). Other parasites are lanceolate or amoeboid and vary in size. The rings have a diameter of 1—2 μ. Elongated forms may be 0.7—1 μ in length and about 0.3 μ in width.

As in other species of this genus, each specimen divides into four lanceolate specimens, arranged in the form of a cross (Fig. 2). Two consecutive divisions of the nucleus precede protoplasmic division.

In the young parasites the nuclei of all four elements forming the cross are placed, in general, near the center of the cross, or are slightly displaced toward the middle of the parasite.

The ring-shaped individuals are the more mature ones. As they become older, they grow in size, the nucleus becomes elongated and finally divides into two. A second division follows very soon afterwards. The newly formed nuclei may be contiguous or situated at the opposite sides of the ring. Sometimes one of the newly formed nuclei of the first division divides before the other; this explains why *Nuttalliae* with three nuclei may be encountered.

B. FELDMAN-MUHSAM: On *Nuttallia adleri* of *Meriones tristrami*

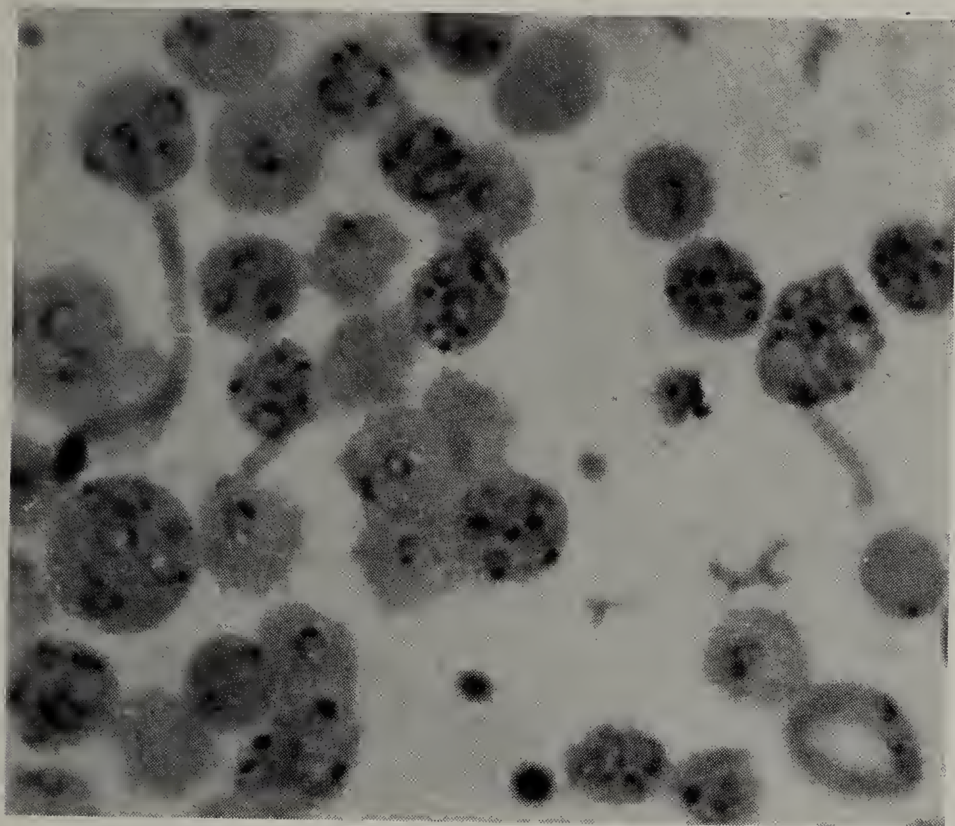

Fig. 1. *Nuttallia* in blood smears of *Meriones*.

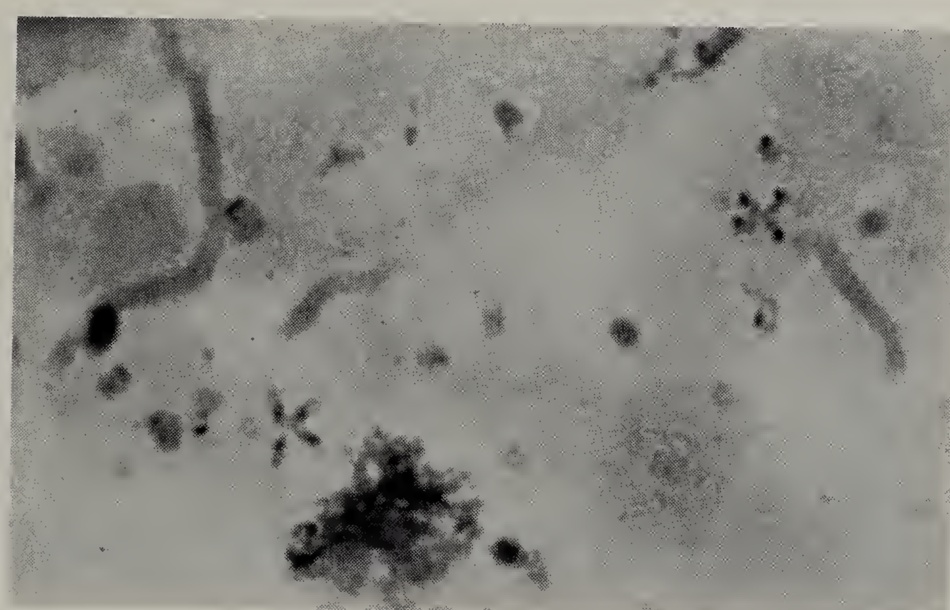

Fig. 2. *Nuttallia* arranged in the form of a cross, immediatly after division.

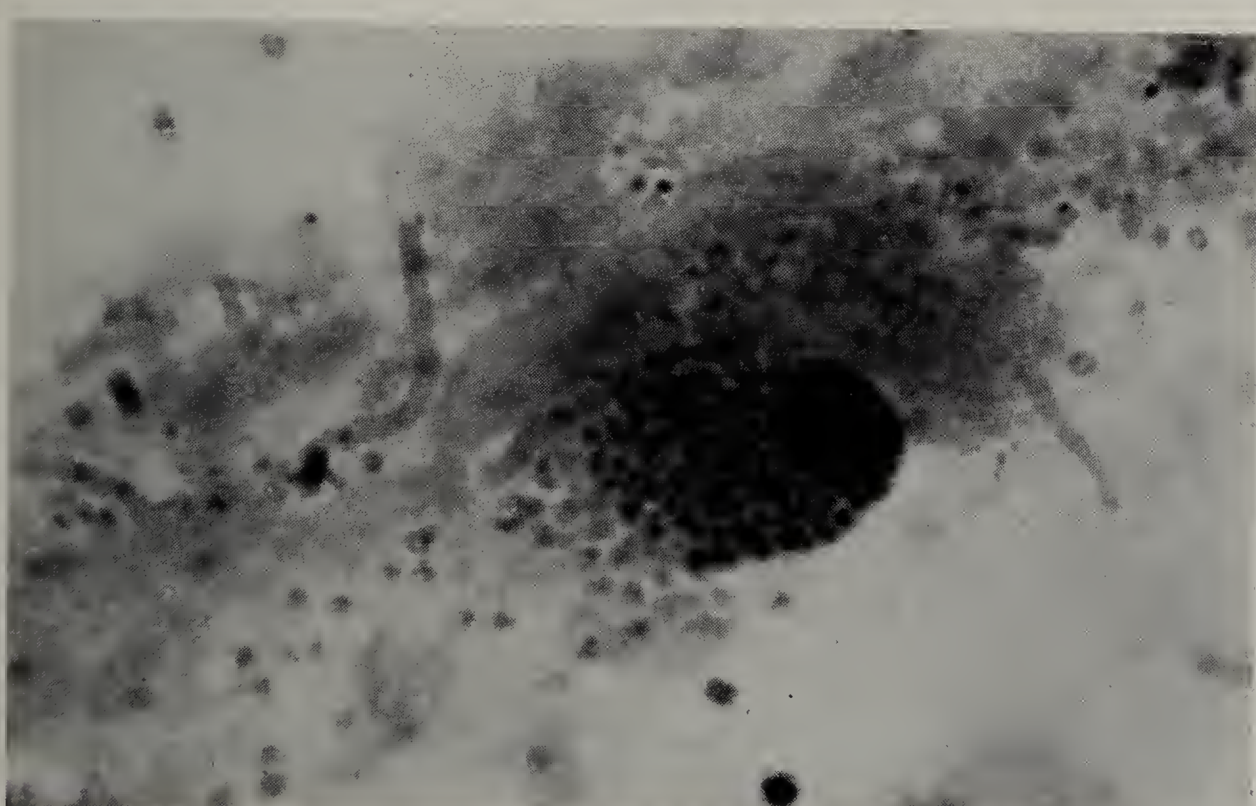

Fig. 3. *Nuttalia* in salivary gland of ticks (smear)

Occasionally band forms stretching across the whole diameter of the infected erythrocyte are found. They are not seen in many infected animals, but are numerous in the few jirds in which they occur. They are common in infected baby mice.

Except in highly infected hosts, the *Nuttallia* is found only in mature erythrocytes, but in heavily infected animals the reticulocytes are also parasitised.

Transmission of *Nuttallia* may be accomplished either by tick bite or by inoculation of infected blood.

In normal, i.e. non-splenectomized jirds, the incubation period, after inoculation, is generally from three to ten days, but it may be longer. The number of parasites in the blood does not increase appreciably. In highly infected animals, the percentage of parasitized erythrocytes does not exceed 16 per cent. After 12 to 16 days the number of parasites in the blood falls, and after five to six weeks, it is impossible to find them in smears. The *Meriones* remains nevertheless infected for at least two years—the longest observation period. In such jirds, splenectomy is always followed by heavy infection.

In splenectomized *Meriones*, the prepatent period varies from 2 to 10 days. The number of parasites rises quickly. In highly infected animals, up to 50 per cent of erythrocytes may be parasitized 11 to 15 days after inoculation. In such cases, 8 to 16 parasites in a single erythrocyte are not unusual; some erythrocytes were found to contain as much as 27. After about a month, the number of parasites decreases. The jird remains infected during its whole life. This latent infection may sometimes be detected only by blood inoculation to a clean, splenectomized animal.

The infection is generally not fatal, neither to the splenectomized animal, nor after splenectomy of an infected animal. The pathogenicity is increased considerably by repeated passage of heavily infected blood through splenectomized animals and fatal infections are produced.

If the infection is transmitted to a splenectomized *Meriones* in the natural way, i.e. by tick bite, the incubation period is longer than in inoculation experiments. The shortest prepatent period observed was 12 days (estimated from the time the infecting nymph commenced to feed) but it often lasts up to 22 days. Infection increases with many divisions, and after ten more days it reaches its climax. Two (out of 19) infected animals died at this stage (25 and 30 days, respectively, after onset of nymphal feed). In the other animals parasitaemia subsided, but they remained infected.

Arthropod transmission

Adler and Feldman-Muhsam showed that the *Nuttallia* of the *Meriones* is transmitted by infected nymphs of *Rhipicephalus secundus* (1952b) fed during the larval stage on infected animals. Transmission is also effected by the nymphs of *R. sanguineus* (Adler and Feldman-Muhsam, 1952c). Experiments were subsequently carried out to determine whether other stages of ticks can also transmit *Nuttallia* (Feldman-Muhsam, 1958). It could be shown that the nymph is the only stage which is capable of transmitting the infection. The injection of ground up, unfed infected nymphs to clean susceptible animals does not produce an infection; this proves that in the unfed nymph, the infective forms of the *Nuttallia* are not yet fully developed.

Information on the cycle of *Nuttallia* in the tick is available only on the first and the last stages. After the infecting feed, most of the parasites are digested in the caeca of the larva. Twelve hours after ingestion, the ingested parasites are still infective to *Meriones* by direct inoculation; six hours later they fail to infect. On the second and third day after engorgement, *Nuttallia* may still be found in smears of the caeca. These *Nuttallia* are larger than those found in the mammal host.

The last stage of development in the tick occurs in the salivary glands of nymphs. Smears of the salivary glands of infected nymphs which had fed for at least a day show that *Nuttallia* develops in the protoplasm of the salivary glands in a compact mass around the nucleus (Fig. 3). Individual sporozoits are very small and have little protoplasm around their nucleus. The intermediate stages of the cycle of *Nuttallia* in the tick still remain to be discovered.

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DISCUSSION

C. B. PHILIP: Did you try infecting adult ticks to see if transovarial passage of *N. adleri* could occur?

FELDMAN-MUHSAM: It was tried but was not successful. Only nymphal ticks transmitted after infection as larvae on infected *Meriones*.

M. COSTA: It would be worthwhile to examine other cave twelling mammals, especially badgers. In Israel it has been found that apparently only the badger is the natural host of *B. persica*.

ASPECTS OF THE ROLE OF TICKS IN THE TRANSMISSION OF KYASANUR FOREST DISEASE

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Manuskript nicht eingelangt

ABSTRACT

The etiological agent of Kyasanur forest disease is a virus closely related to that of Russian spring summer encephalitis, central european tick-borne encephalitis, louping ill and several others of the group B arthropod-borne viruses. The disease was discovered in India and the agent isolated since the last International Entomological Congress. The virus has been isolated from or antibodies demonstrated in a variety of vertebrate hosts including man monkeys, small forest mammals, various domestic animals, and several bird species. Among the arthropods investigated virus has been isolated repeatly from ticks of several genera but mostly from the genus *Haemaphysalis* with by far the greatest number of isolations being from *H. spinigera*. Conflicting evidence has been obtained on the critical point of whether or not the virus passes transovarially to the progeny of infected ticks. On two occasions virus has been from questing and presumably unfed larvae of *H. spinigera*. But in an area where virus was extremely active as

evidenced by repeated isolations from nymphs and adults, larvae of the subsequent generation appearing the following season failed to yield virus although thousands of specimens were processed. The implications of the results obtained for the maintenance of the virus in nature will be discussed.

DISCUSSION

C. B. PHILIP: Is there evidence of inapparent or low grade infection in the indigenous monkey hosts of the ticks?

H. TRAPIDO: There was evidence that some monkeys in nature had become immune.

H. BARNETT: The implication that inapparent infection in KFD was not very common is in contradiction to the numerous reports in the Russian literature of inapparent infection with the various R. S. S. E. viruses in the Soviet Union. Further, the possibility was pointed out that virus transovarially transmitted to larvae might be modified to a immunogenic form from a pathogenic form during this process. Is check such a possibility, it would be necessary to challenge those mice which had survived inoculation with larval pools, to determine whether or not they possessed any immunity.

C. E. HOPLA: A similar instance of one pool of infected larvae was reported by Calhoum and associates in relation to ticks, and Tularemia in Arkansas. To my knowledge (Dr. Philip can check me on this) this is the only pool of larvae that have been reported infected with Tularemia organisms.

H. TRAPIDO: In reference to your one pool of infected larvae, I fully agree that when one is working with large numbers of ticks in the field, it is easy to place one or more nymphs in with the larvae. However, I would like to point out that a larva, nymph or adult which has been attached to a host up to 36 hours and detached of its own free will (for one reason or another) most likely will show no signe of engorgment.

W. W. MACDONALD: Since there was only one isolation from larvae, may it not be that very few virus particles are present in the larvae and that there might be detected by blind passages, which were, as you mentioned, not carrier out?

IXODES SCAPULARIS AS A VECTOR OF TULAREMIA ORGANISMS IN THE SOUTHERN UNITED STATES

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Introduction

In reviewing the epidemiology of tularemia in the southern United States, it is soon evident that the majority of human cases are tick-borne. Furthermore, the majority of these cases occur from April through July. The work of Hopla (1953, 1954, 1955, 1960) and by Calhoun (1954, 1955, 1956) have shown the relationship of *Amblyomma americanum* and *Dermacentor variabilis* to this particular type of transmission during these months. However, during the last three months of the year and on into January, there is a sharp decline in the number of reported cases, most of them due to animal contact, especially with the cotton tail rabbit (*Sylvilagus floridanus*). Certain of the cases, however, are clearly tick-borne. The results of the study reported here is concerned with the elucidation of the tick vector during this particular time of the year.

Four species of ticks occur during October through January. However, because of the restricted host habits of two species and the extremely low numbers of the third, only one species, *Ixodes scapularis*, appears to be important, especially since it readily feeds upon man when an adult. For this reason and due to the fact that certain evidence indicated it was the actual tick concerned in some case histories, *I. scapularis* was selected for the various experiments undertaken in this study.

Materials and Methods

The original stock of ticks reared for the quantitative studies were obtained near Mena, Polk County, Arkansas. The white laboratory rabbit was used as the experimental animal for feeding the ticks inasmuch as the guinea pig was found to be an unfavorable host. With this exception, the methods used were essentially those reported by the author in previous publications. It is perhaps well to indicate that *I. scapularis* is an especially difficult tick to rear and must be retained at a high relative humidity.

Results

Quantitative studies showed that this tick was readily infected inasmuch as 70 per cent of the 300 ticks examined proved to infected. The average number of organisms per infected tick was established at 5.5×10^6. The number of organisms varied considerably from this, ranging from 4.1×10^4 to 1.2×10^8. This compared reasonably well with earlier studies dealing with *A. americanum* and indicated that it should be a good experimental vector.

A large number of larvae were infected by feeding upon an infected host and a series of them placed upon rabbits for the nymphal and adult feedings. A fully virulent strain of *Bacterium tularense* was used (LD_{50} of $10^{-9.6}$ for mice) inasmuch as death would be produced in the host in nearly all instances, even when as few as 3 or 4 organisms were transmitted. This is known as the Sm strain of *Bact. tularense*. The results of this study indicated that *I. scapularis* was able to transmit the organisms from one stage to the next and once infected would remain so for the rest of its life. Small scale expermints dealing with attempted transovarian transmission was not successful.

During October of 1955, 42 unfed females and 28 males were collected in the vicinity of Smithville, McCurtin County Oklahoma. The ticks were divided into 7 equal lots with regards to both sexes and each lot placed upon a rabbit. Two of these rabbits became ill, one on the ninth day and the other one on the thirteenth day and were dead on the 12th and 13th days respectively. All ticks had engorged by the 11th day and were stored in tubes designed to give a relative humidity of 90 per cent. Autopsy, Gram's stain, plate cultures, and subsequent animal inoculations established that the organism which had been transmitted by the ticks was *Bact. tularense*. LD_{50} titrations of once passaged material established that in both instances the organism was one of high virulence since the titre in various susceptible laboratory animals ranged at least above a dilution of 10^{-8}.

In May of 1956 a "skink" was found infested with 6 nymphs which were subsequently identified as *I. scapularis*. The "skink" was retained in the laboratory until the nymphs had completed feeding and detached. These ticks moulted into adult ticks 4 weeks later and were placed upon a rabbit for the adult feeding approximately 2 weeks after the nymphal moult was completed. This animal relatively ill on the 10th day after the ticks had attached and was dead on the 13th day. Procedures carried out as outlined in the preceding paragraph established the cause of death in the rabbit to be due to *Bact. tularense*. The LD_{50} titration showed the organisms to be of moderate virulence, with a titre of $10^{-6.7}$ for white mice.

The latter isolation is somewhat confusing inasmuch as cold blooded vertebrates are not generally considered important in the ecology of tularemia. However, lizards and skinks are the most common host of the immature stages of *I. scapularis* in the area I have studied. For example, of 235 collection records, 91 per cent are from this group of animals. It is not known whether these hosts are actually prefered or if it is simply a problem of availability. Extensive populations of small rodents which could serve as hosts for the immature stages, are not readily available. It does not seem reasonable (for various reasons) to assume that *I. scapularis* is a vector of major importance.

For example, it does not occur in the large numbers that are characteristic of *A. americanum* in the same area earlier in the year. Insofar as known, it attacks man only during the adult stage, thus giving it but one chance to act as a vector.

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THE ENIGMA OF TICK PARALYSIS IN NORTH AMERICA

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Introduction

Irene Pavlich, aged two years, got up April 29th at 5 a.m. and had breakfast with her father. Later she complained that her legs were tired; the next day she had difficulty standing and the second morning there was marked weakness in her legs and arms. Two engorging ticks were found and removed from the back of her head. Six hours later she sank into a coma; her breathing became difficult and in spite of artificial resuscitation she died early that evening (1).

Such is the gravity of tick paralysis in North America. The clinical symptoms consist of an ascending motor paralysis advancing over a period of two to ten days. Reflexes become absent and, finally, there is inability to swallow and breath. Recovery usually follows removal of the tick, its speed depending largely on the degree of paralysis. Mild symptoms of ataxia may disappear within an hour; cases verging on bulbar paralysis may take several days to recover (2, 3, 4). In rare instances symptoms of muscle weakness have lasted for several weeks (5, 6).

The symptoms in domestic animals are similar to those in humans. The posterior region is the first to be affected and causes a swaying of the hind quarters as the animal walks. It is finally unable to stand, and, if unattended, ultimately dies from respiratory failure. In sheep and dogs paralysis occasionally disappears while a group of ticks is still feeding; commonly, it remains until the last tick is removed.

In groundhogs, guinea pigs, and hamsters the paralysis is not so marked and may be accompanied by salivation, conjunctivitis, and loss of voice. Nevertheless, these animals frequently fail to recover following the removal of the ticks (7).

Epizoology

Host susceptibility. It appears that among the large domestic animals, man, cattle, horses, sheep, and dogs are particularly susceptible and pigs and cats less so, or not at all (8). Wild animals appear to be rarely affected naturally, there being only records in 8 buffalo (9) and a blue fox (10). Marmots, groundsquirrels, packrats, guinea pigs, and hamsters, have been shown to be susceptible to laboratory infestations of *Dermacentor andersoni* Stiles (11).

Host resistance. Two host factors may mask the potential prevalence of tick paralysis. The first, an acquired resistance to tick feeding which has been observed during repeated

laboratory infestations of sheep with adult *D. andersoni*, could explain instances where imported animals have suffered more from tick paralysis than have local ones and would account for the higher incidence of paralysis in yearling cattle than in older cows (12, 13). The second factor concerns a natural variation in the resistance to paralysis within certain species of hosts. Although some dogs and lambs have been paralysed by initial infestations of solitary British Columbia *D. andersoni* females, others have been unharmed by as many as fifty. That this variation appears to reside in the host and not in the tick is evident from the high incidence of paralysis caused by solitary *D. andersoni* from the same region on marmots and humans (8).

Host immunity. Immunity to tick paralysis itself has been shown to be absent in at least sheep (14, 15), groundhogs (8) and hamsters (11).

Tick virulence. Indications of any long term periodicity in the prevalence of the disease are difficult to detect, mainly because human cases each year are relatively few and because once ranchers have suffered from an outbreak involving hundreds of animals they remain very conscious of tick control for some years. The apparent absence of the disease during early years of ranching in British Columbia (1860 on) is interesting. Although there are occasional records of paralysis in sheep and cattle since 1910 (16, 12), the first major outbreak in cattle did not occur until 1930 when a hundred animals were paralysed (17). Subsequent outbreaks in this province in 1935 (13), 1944, and 1957 (18) involved 200, 44, and 320 paralysed animals.

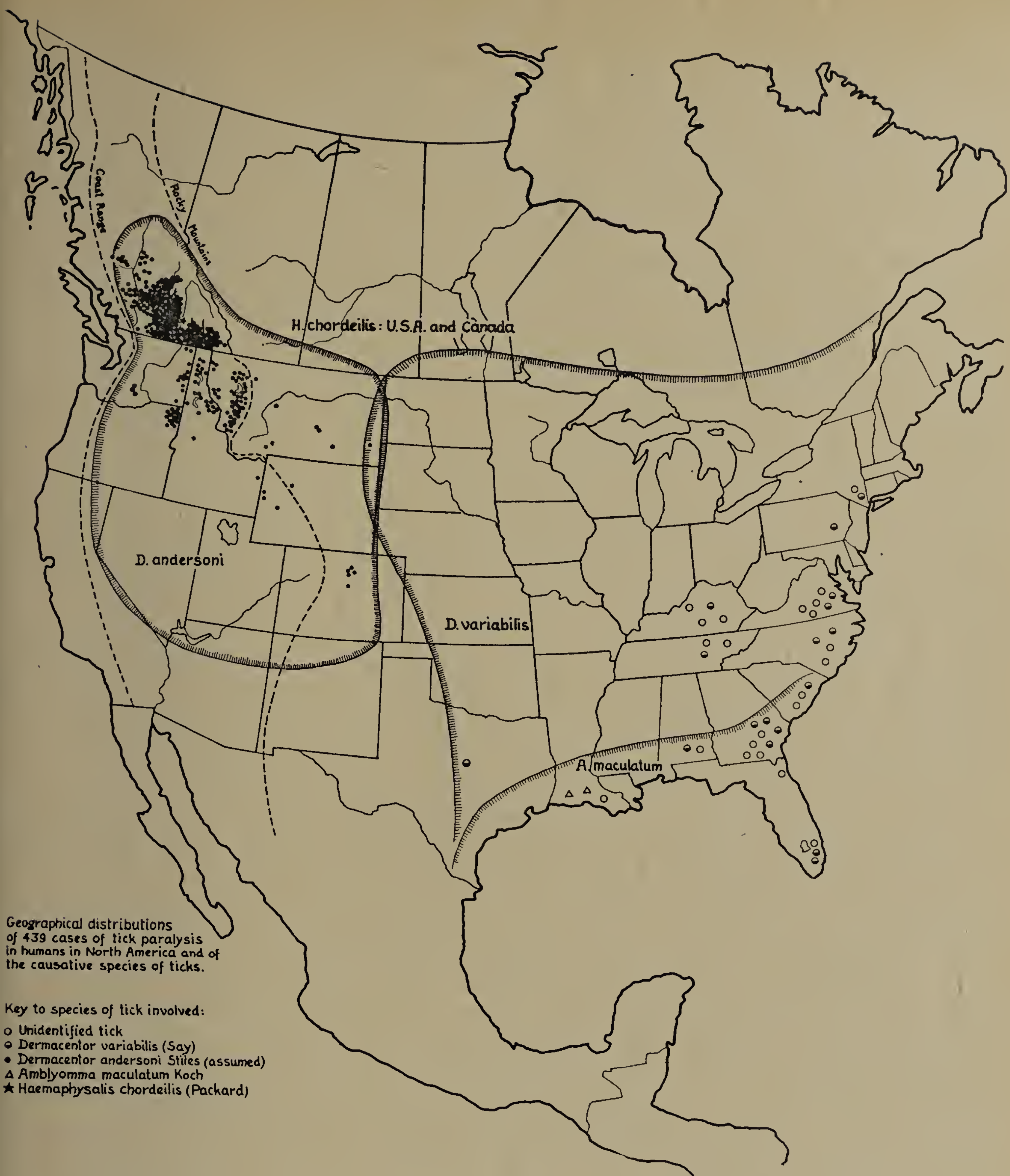
Possible evidence of a change of virulence in tick paralysis has appeared in the recent observation that guinea pigs are now about fifty per cent susceptible to paralysis by *D. andersoni* from British Columbia and Montana (11, 7). Formerly, during the course of Rocky Mountain spotted fever studies, thousands of similar ticks were fed on these animals with only rare instances of paralysis (19, 2).

The distributional aspect of *D. andersoni* virulence is more obvious (20). Although the distribution of the tick extends as far south as Colorado and California, and as far east in Canada as Saskatchewan, paralysis is rarely produced east of the Rocky Mountains or south of the 45th parallel.

Etiology

The relationship of tick attachment to host paralysis. It appears that only the female tick produces a true ascending paralysis. She must first have a blood meal for about five days, but she need be neither mated nor nearing repletion. Indeed, in the case of humans and marmots, a tick may be but a tenth of its final weight and engorging very slowly, yet still produce paralysis. At Kamloops it has been found that up to three dozen ticks that have already fed on one host for five days may paralyse new hosts (groundhogs) within ten to 24 hours of the transfers. Up to a point, this latter interval tends to become shortened by successive transfers (7). In one instance when a marmot was mass infested with a hundred such pre-fed ticks, paralysis was still not produced until four hours had elapsed (21). It does not matter on what portion of the body the tick feeds, nor is the age or size of the victim a factor. Men, as old as 74 years, and cattle, weighing up to 1000 lbs. have been paralysed by a solitary tick.

The mechanism of paralysis in the host. Recent research on tick paralysis in North America has centered mainly around studies on effects produced on the nervous system. It was first shown that a peripheral neuromuscular block was present in tick-paralysed dogs and lambs since electrical stimuli applied through motor nerves failed to give contractions in muscle that responded to direct stimulation (22, 23). This finding was confirmed in paralysed marmots (24). However, the degree of the block was found to be relative to the extent of paralysis and even in advanced cases neuromuscular impairment was only 77 to 92 per cent of that in the normal animal (25).



At first it was thought that conduction in the motor and sensory pathways of the paralysed animal was normal (26). Later studies on paralysed dogs showed a defect in the conduction of the motor nerve fibers, particularly at their fine terminal endings (27). It was next shown that in marmots sensory, as well as motor, fibers appeared to be affected, and that, furthermore, there was a depressing action on heart muscle and the central nervous system (28). Spinal cord dysfunction in marmots has been attributed to a definite and selective impairment of the monosynaptic reflex pathway with the suggestion that it, too, might be blocked through its fine afferent fibers (25).

The polysynaptic pathways, on the other hand, have appeared to remain mainly functional, possibly due to their less extensive branching (25). There is, in the paralysed animal, normal functioning of the vagus nerve and the cervical sympathetic system, respective stimuli causing cardiac slowing and nictitating response (29).

The observation that rapid close intra-arterial injections of acetylcholine caused tick-paralysed muscle to contract (26) has been complemented by the finding that little or no acetylcholine was liberated upon nerve stimulation of muscles in paralysed marmots and dogs (24, 27). It has also been demonstrated that the nerve tissues of paralysed animals are still capable of synthesizing acetylcholine (28, 27). Paralysis thus appears to be explained by a failure of nerve impulses to pass through the nerve's finer endings and thus release acetylcholine.

Attempts to demonstrate the presence of the toxin, either within the tissues of a paralysed host or within the tick have been singularly negative. Blood exchanges between paralysed and normal marmots have failed to bring about any marked changes in the animals (30), and perfusions of heparinized plasma from a paralysed dog produced no block in an isolated rat diaphragm (22). Injections of secretion collected from the mouthparts of engorging ticks into animals susceptible to paralysis have been equally unrewarding (31). Even an aggregate of ¼ cc. of this secretion, and the emulsion of 58 pairs of tick salivary glands, injected intradermally and subcutaneously into marmots over periods of 7½ and 24 hours, respectively, failed to produce any indication of paralysis (21). The one instance of death in a marmot from a rapid subcutaneous injection of crushed salivary glands (8) is now believed to have been due to shock since a repetition of the experiment using three times the first dosage per body weight failed to bring about paralysis.

In vitro nerve-muscle experiments have been equally negative, and perfusions of tick glands and their secretions, whether alone or brewed with blood, or with skin brei, have failed to have any effect on frog gastrocnemius, and rabbit and marmot lumbrical preparations (21).

Incidence of tick paralysis in North America in relation to species of ticks.

By far the greater portion of tick paralysis cases in North America are caused by *D. andersoni* females. There are over 350 records of recovery in humans from paralysis by this species. In addition, there have been 43 deaths from advanced respiratory involvement through failure to discover or to remove the tick in time.

The next most important species of tick causing paralysis in North America is *D. variabilis* (Say). Paralysis was not recorded from presumably this species until 1938 (32). Since then it has accounted for at least 38 cases of human paralysis, including one fatality. Contrary to *D. andersoni*, *D. variabilis* appears to be more virulent in the southern portion of its range; in Florida it is not unusual for some veterinarians to treat as many as 50 cases of tick paralysis in dogs in a year (33). Although this species of tick is very common in eastern Canada there are no records of paralysis by it there either in humans or domestic animals.

Paralysis by three other North American species of ticks has been produced also on very rare occasions. *D. occidentalis* Marx has been reported to have caused paralysis in cattle in two outbreaks in Northern California (34, 35). A non gravid female of *Amblyomma maculatum* Koch produced paralysis in a seven year old girl (36) and two partially fed nymphs of the same species caused ataxia in a 12 year old boy (37). Both cases occurred in Louisiana. This is the only record in North America of paralysis having been produced by nymphal ticks. *Haemaphysalis cinnabarina* [= *H. chlordeilis* (Packard)] has only once been recorded as producing paralysis, but in this instance one engorged female killed a ten year old child (38). There are no records of an ascending paralysis being caused by the males of any of the above species.

The agent causing tick paralysis in North America remains unknown. There is little doubt that its ultimate classification will contribute in some way to the knowledge of metabolism within the tick and of nerve transmission within the host. Perhaps, like curare, the toxin will even find a place in practical medicine.

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DISCUSSION

QUESTION: Have you considered the possibility that the paralysis may be caused by a tick-borne organism that resides within the tick and, becoming activated, produces toxin which becomes liberated into the host?

J. D. GREGSON: Yes, I think that this is possible, providing that the activated organism remains within the tick, thus permitting the rapid recovery in the host following the removal of a causative tick.

D. W. JENKINS: What is the possibility that tick paralysis is caused by an toxin producing organism which lives as a symbiote in the tick? If a rickettsid or bacterial agent were present which produces a toxin injected by the tick into the vertebrate host, this might explain the local distribution of paralysis producing tick within the range of the tick. If a rickettsial organism were present, a required reactivation phenomenon would explain the delay in causing paralysis after the tick has attacked. I am referring only to tick transmission of the toxin and not the rickettsial or bacterial organism to the vertebrate host.

FACTORS LIMITING THE DISTRIBUTION OF IXODES RICINUS IN NORWAY IN RELATION TO THE OCCURRENCE OF TICK-BORNE DISEASES

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For about 60 years *Ixodes ricinus* has been known as the vector of piroplasmosis in Norway, causing about 1200 to 2000 cases of the disease every year. In recent years the species has been found to transmit other diseases as well, such as the "tick-borne fever" and "scrapie" of sheep—probably also "louping ill". Among diseases affecting man, *erythema chronicum migrans* connected with meningitis, is of some importance. *Pasteurella tularensis* has been isolated from ticks collected on a sick hare in south Norway; and it seems likely that human infections of "louping-ill" may occur. The tick has been suspected of transmitting *sclerosis multipla* as well, but with our present knowledge of the ecology and distribution of the species, that seems rather unlikely.

Ixodes ricinus has a quite peculiar distribution in Norway. It is found along the coast from the Oslo-fjord to Brönnöysund (about 66° N)—the northernmost locality where the species has been recorded—about 5° north of its main northern border in Sweden, Finland and the USSR. With this distributional pattern, *I. ricinus* belongs to a large group of so called "coast" or "Atlantic" animals and plants. In a recent work Fægri (1960) lists some 150 species of higher plants as belonging to that biogeographical group. Representatives are found among cryptogames and different groups of terrestrial animals as well. The deer (*Cervus elaphus*) may be the best known example.

Some of the coast species are confined to the extreme western part of the coast, while others go deeper in the region of the great fjords. Many of them follow the coast eastwards to the Oslo-fjord or even to the surroundings of Oslo. Those which are restricted to the outermost line of islands will usually be found in the southwestern part of the country only; while those occurring in a broader zone in the southwest will usually follow the coast both eastwards and a shorter or longer distance to the north.

This pattern of distribution can very easily be correlated with some isotherms, especially with winter isotherms, which more or less follow the coastline. That does not mean, however, that temperature is to be considered the chief limiting factor for the distribution of the flora and fauna concerned. In fact, *I. ricinus* may serve as an example of a species whose distribution is governed mainly by the *humidity* of its surroundings.

British investigations have shown very clearly the importance of high relative humidity in the ecology of *I. ricinus* (see *e.g.* Campbell 1949 and the references given there). A percentage of 85 to 90, or even more, is necessary for normal development, breeding and host-seeking activity. It is not possible to map the distribution of humidity percentages in such a way that it could be used in a discussion of the distribution of *I. ricinus.* But a comparison of its distribution with some climatological factors which influence humidity may be useful. From fig. 1 it is evident that high precipitation is of great importance. One should then bear in mind that this *southern* species in our fauna is restricted to those areas in southern Norway which have the *coolest* summers. One even gets the impression that it is prevented from spreading to other neighbouring areas where the summers are usually considerably warmer. The occurrence of the species

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Right: Fig. 1. The distribution of *Ixodes ricinus* in Norway. Black dots: localities from which specimens have been obtained. To the coastal side of the dotted line the tick and piroplasmosis are common in suitable habitats. Dots "inside" the line represent scattered occurrences. In the climagraphs mean temperatures (horizontal axis, origo at 0° C) are plotted against mean precipitation (vertical axis, origo at 100 mm) for every second month.

Sandstad
Balestrand
Sytteland
Klepp
Flekkefjord
Kragerø
Oslo
Eidfjord
Lærdal
Steinkjer
200
10

in the cooler districts with a high precipitation compared with its lacking in the warmer and dryer areas, gives strong indications of the importance of the humidity factor. In the border districts around the Oslo-fjord, where the tick is found in a very narrow zone along the coast or sporadically in suitable localities only, the climagraphs are of an intermediate type, or they are (as the Oslo climagraph) certainly not representative of the localities where the tick is found.

In its chief area of distribution in western Norway, the tick mainly occurs in two seemingly quite different habitats. The farmers distinguish between "heather-ticks" and "alder-ticks" and believe them to be different species. The "heather-ticks" are found on pastures in the westernmost coastal districts. A short vegetation of heather, bracken and different grasses and sedges covers the pastures in wet hollows and furrows between low stony hills. Both in the lower parts and on the hillsides the soil consists of a wet, swampy mat that retains the water and keeps the moisture in the vegetation layer near to saturation point. The vicinity of the open sea also tends to keep the moisture high, even if the precipitation is not especially high in those outer areas.

In the fjord districts, the "heather-tick" gives way to the "alder-tick". The heaviest infestation is found on hillsides covered with deciduous trees and shrubs and with a very wet soil even in steep places. This is the zone of highest precipitation. Patches of grassland, used as pastures, are found as small openings bordered by dense shrubs or woods with *Alnus glutinosa* as the dominating species. Very often the cattle will have to press their way through such bushy belts in order to move from one grassfield to another.

These two habitats have in common the high relative humidity within the vegetation layer. Localities are known where ticks have been found in abundance in vegetation quite different from what has been described here; but those localities stand out clearly as exeptions and they too show the same common factor, a layer with high humidity.

On the other hand, it is a common experience among veterinarians, agriculturists and farmers in the tick districts, that whenever pastures are improved by cutting down alderbushes or draining the soil, tick infestation is reduced very markedly—sometimes to nil. The occurrence of piroplasmosis is also reduced considerably on farms where such measures are taken.

Another series of observations will make clear the importance of tick ecology in relation to the occurrence of tick-borne diseases. Whenever cattle or sheep are brought from the inner districts to the coast they will run a heavy risk of getting respectively piroplasmosis or "tick-borne fever". This was experienced by the farmers long before the time of modern veterinary science and is reflected in the name they gave to both diseases: "sjodogg", which means a sickness caused by the sea-air.

On the other hand, it repeatedly happens that ticks are carried with cattle, sheep or goats outside their normal area of distribution. In some cases, engorged ticks have left their hosts on suitable pastures, they may then moult to the next stage on the spot, thus causing a few scattered cases of piroplasmosis among the cattle from the surrounding farms. All along the old routes where cattle or sheep are driven from West Norway to the eastern part of the country for sale, or from coastal districts to summer pastures in the mountains, cases of tick transport have been recorded (fig. 2). But in none of these cases have the ticks been able to establish a population in the new locality.

The question remains whether the humidity factor can explain the main features in the total distribution of *I. ricinus*. A map very roughly indicating the total distribution of the species (Tambs-Lyche 1943 p. 521) has some interesting features in common with a recent map of some European floristic regions (Fægri 1960, p. 23 after Troll). The eastern border of *I. ricinus* shows a striking similarity to the eastern border of Troll's sub-atlantic climatype. It also in the main coincides with the easternmost influence of

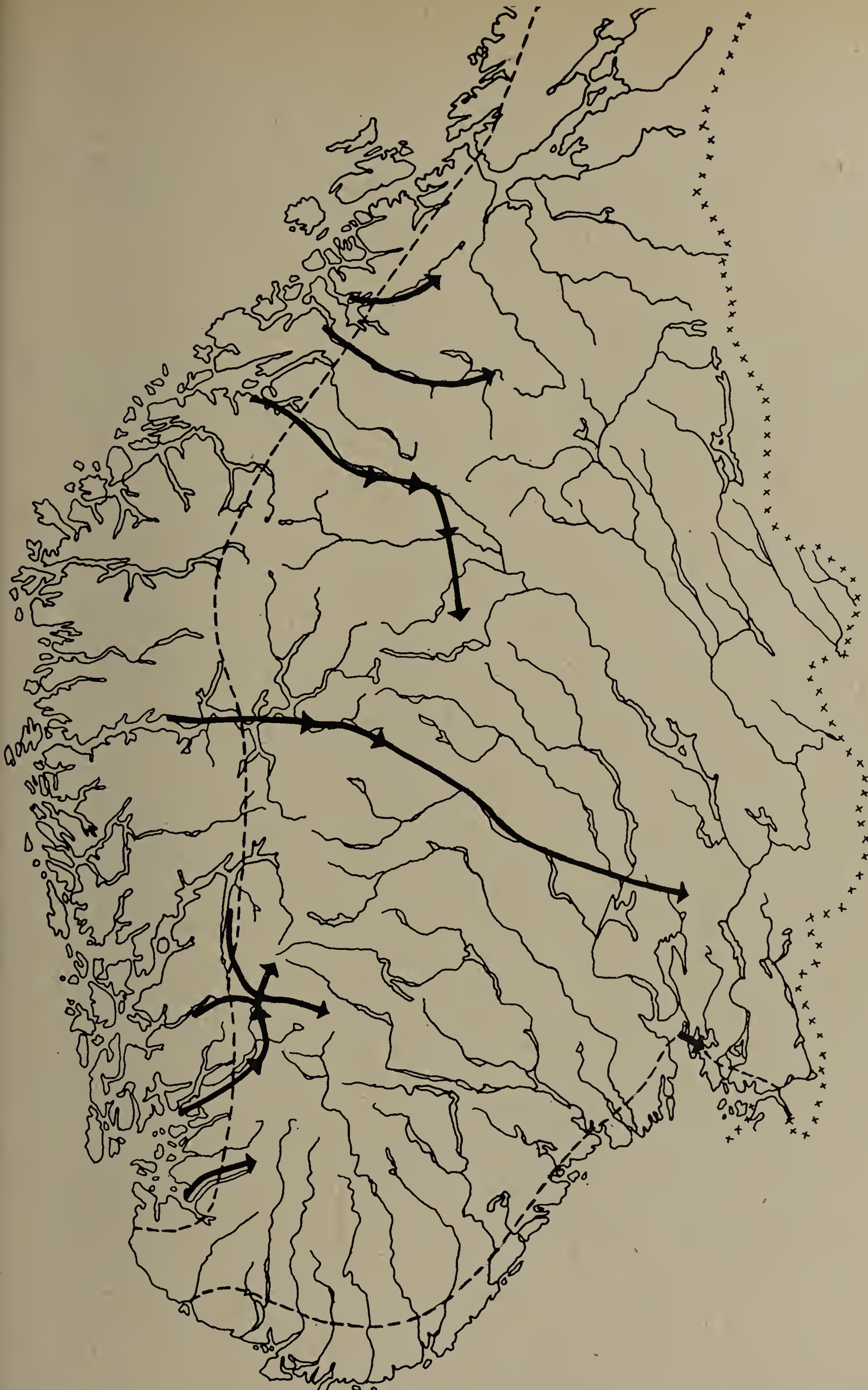

Fig. 2. Transport of *Ixodes ricinus* outside its main area of distribution. The arrowheads indicate places from where ticks have been reported.

the wet Atlantic summer winds (compare Alt 1932). There seems, however, to be insufficient information available as to the ecology of *I. ricinus* in Central and South-eastern Europe. It would be of great interest to compare the ecology of *I. ricinus* with that of its near relative *I. persulcatus*. Both species are known to be vectors of encephalitis-viruses, but a review of the medical litterature from a zoological point of view gives the impression that there may be some host-specificity between viruses and ticks. It would be worth while to see if *I. persulcatus* transmits the Russian springsummer-encephalitis, while *I. ricinus* is the vector of "louping-ill".

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DISCUSSION

J. A. CAMPBELL: Dr. Tambs-Lyche has emphasised the climatological factors which limit tick distribution in Norway and suggests that ticks are ubiquitous within the climatologically favourable area. In Britain we find a marked discontinuity of distribution which cannot be explained solely on the basis of climatological differences. It is true that the water requirements of this species are high, and microclimatic differences in respect of this factor are sufficient to account for the absence of ticks from the well drained grazings of S. E. England, often in a chalk substratum and always dry in summer. On the other hand they are generally distributed throughout the western and northern sheep grazings of Great Britain where the underlying palaeozoic rock are often covered with glacial drift and peat and are usually poorly drained. Nevertheless, with this zone the discontinuity of distribution is still marked and the presence or absence of ticks is not directly related to the presence or absence of favourable microhabitats. A classical example is in the valley of the river Tweed in Southern Scotland where the hills south of the river are virtually tick-free. True this boundary appears recently to the breaking down and ticks are appearing on the northern slopes of the valley. The district is known, however, to have been tick infested for at least a hundred years—how can we then explain the freedom from tick of the northern slopes until the last ten years ago?

Apart from those areas where red-deer or blue bures are numerous the only significant tick (*I. ricinus*) populations of the British Isles are maintained by domestic stock, namely sheep and cattle. Now even within a given sheep grazing the distribution of ticks is very discontinous some areas carrying heavy populations and others few or none. To some extent these differences are related to the pattern of distribution of vegetation, some plant communities being much more heavily infested than others. This in turn is related to the host potential on them, since sheep congregate densely on different plant communities at different times of the year. Those which are heavily grazed in spring—the culture season of the tick in S. Scotland—carry the large tickpopulations. I have doubt that this distribution within a single grazing is due to host movements and is not the result of a differential mortality on different types of vegetation since experimental plantings of ticks reveal us differences in mortality rates twelve months later after the complection of a moulting cycle.

I suggest, therefore, that in addition to climatological factors, whose effects are relatively simple and obvious there are other very important factors which leed to the very striking discontinuity of distribution of ticks in Britain at least. The pattern of host movements is one, but I believe there must be others whose nature we have not yet discovered.

UNTERSUCHUNGEN ÜBER BRUCELLOSE IN BULGARIEN
und
UNTERSUCHUNGEN ÜBER TULAREMIE IN BULGARIEN

P. PAVLOV

Manuskript und Abstract nicht eingelangt.

EINE NEUE SPIROCHAETEN-ART, BORRELIA TILLAE ZUMPT & ORGAN, AUS ORNITHODOROS ZUMPTI HEISCH & GUGGISBERG UND AUS WILDRATTEN IN SÜDAFRIKA

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Im Jahre 1952 gelang es zum ersten Mal bei Port Elisabeth in Südafrika in dem Nest einer Wildratte (*Rhabdomys pumilio*) eine *Ornithodoros*-Art zu entdecken, die offenbar als typischer Parasit dieses Nagers anzusehen war. Diese Art, von der 3 ♀♀, eine Nymphe und 2 Larven vorlagen, wurde dann im folgenden Jahr von Heisch und Guggisberg als *O. zumpti* beschrieben. Es gelang uns damals nicht, aus den Zecken Spirochaeten zu isolieren.

Im Oktober 1959 erhielten wir weitere Exemplare dieser Zecke, und zwar dieses Mal aus dem Nest der Ratte *Otomys saundersiae*. Diese Zecken wurden an weißen Mäusen gefüttert, welche nach wenigen Tagen eine hohe Infektion mit Spirochaeten zeigten und den typischen Verlauf eines Rückfallfiebers. Die serologische Untersuchung ergab eine nahe Verwandtschaft mit einem Stamme von *Borrelia duttoni* aus dem nördlichen Betschuanaland, jedoch waren im Komplementbindungstest Unterschiede feststellbar, die uns veranlaßten, diesen aus *O. zumpti* isolierten Spirochaetenstamm als neue Art zu betrachten und als *Borrelia tillae* Zumpt & Organ in die Literatur einzuführen. Es wurden dann in der weiteren Umgebung von Port Elisabeth weitere Untersuchungen durchgeführt, und es gelang, einen weiteren Stamm aus Exemplaren aus *O. zumpti* zu isolieren und 6 Stämme von Spirochaeten aus dem Gehirn von 3 Ratten der Art *Rhabdomys pumilio* und 3 weiteren Exemplaren von *Rattus natalensis*. Insgesamt wurden Gehirnemulsionen von 53 Wildratten in weiße Mäuse injiziert. Die serologische Identität dieser 7 weiteren Isolationen mit dem ersten Stamm wurde bisher noch nicht geprüft, aber es dürfte kaum ein Zweifel bestehen, daß sie alle zu *B. tillae* gehören.

Weitere Arbeiten mit *Borrelia tillae* im Vergleich zu typischen Stämmen von *B. duttoni* werden z. Z. im Südafrikanischen Institut für medizinische Forschung durchgeführt. Insbesondere gilt es festzustellen, ob und unter welchen Umständen *B. tillae* durch *O. moubata* übertragbar ist, und ob es gelingt, Rhesus-Affen und evtl. auch Menschen zu infizieren. Es ist durchaus möglich, daß *B. tillae* die Mutterform von *B. duttoni* darstellt, und daß der Erreger des klassischen afrikanischen Rückfallfiebers durch Adaption an *O. moubata* entstanden ist. Der eigentliche Wirt von *O. moubata* ist nach heutiger Ansicht nicht der Mensch, sondern das Warzenschwein, bei dem *O. moubata* in vielen Teilen Afrikas, einschließlich Südafrikas, ein normaler und häufiger Parasit

ist. Aber diese „wilden Tampans“ sind niemals mit Spirochaeten infiziert gefunden worden, und auch das Warzenschwein verhält sich völlig refraktär experimentellen Infektionen gegenüber. Dagegen haben sich die Wildratten in Südafrika zu einem hohen Grade als infiziert erwiesen und sie zeigen eine langandauernde, jedoch normalerweise nicht lethal verlaufende Parasitaemie mit *B. tillae* und, experimentell, auch mit *B. duttoni*. Das trifft ganz besonders für *Rattus natalensis* zu, der als typische Wildratte häufig und regelmäßig auch in die Hütten der Neger eindringt (vgl. Zumpt, 1959). Es ist daher sehr naheliegend, daß nichtinfizierte, durch jagende Neger aus den Warzenschweinbauten verschleppte Zecken gelegentlich an solchen Ratten saugen und eine Infektion mit *B. tillae* erwerben. Es ist durchaus nicht notwendig, daß eine Adaption der Spirochaeten regelmäßig oder auch nur häufig stattfindet. Es genügt, daß nur selten oder nur einige Male die normale Barriere unter bestimmten und vielleicht ungewöhnlichen physiologischen Bedingungen niedergebrochen sei, um die Entstehung der an *O. moubata* adaptierten *Borrelia* aus den Rattenspirochaeten zu erklären.

LITERATUR

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EPIDEMIOLOGISCHE UNTERSUCHUNGEN ÜBER DIE VERBREITUNG DER FRÜHSOMMER-MENINGO-ENCEPHALITIS IN NIEDERÖSTERREICH

H. MORITSCH

Seit 1927 wird im Bezirk Neunkirchen (Niederösterreich) beobachtet, wie vor allem in den Monaten Mai bis Juli die Frühsommer-Meningo-Encephalitis (FSME) unter der Bevölkerung (ca. 100.000 Einwohner) endemisch auftritt.

Diese virusbedingte Infektionskrankheit des Zentralnervensystems (ZNS) wird klinisch als Meningitis, Meningo-Encephalitis oder Meningo-Encephalitis mit Paralysen manifest, wobei diese klinische Verlaufsform vor allem vom Alter der Patienten abhängig zu sein scheint; da diese Erkrankung sowohl klinisch als auch pathologisch-anatomisch der Poliomyelitis sehr ähnlich ist, kann sie mit Sicherheit nur durch gezielte virologisch-serologische Untersuchungen abgegrenzt werden.

Diese Untersuchungen wurden seit 1956 im Bezirk Neunkirchen regelmäßig vorgenommen. Dabei gelang es in 54% aller Fälle von Infektionskrankheiten des ZNS eine FSME zu identifizieren.

Jahr	Gesamtzahl aller Fälle mit Infektionen des ZNS	davon FSME
1956	80	44
1957	53	31
1958	29	14
1959	25	13
	187	102 (=54 %)

Die Erkrankung tritt gehäuft vor allem in den Frühsommermonaten mit einem Maximum im Juli auf.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Total
Frühsommer-Meningo-Encephalitis	1	—	1	2	14	23	32	10	9	5	4	1	102
andere Infektionen	6	—	3	3	1	1	11	21	11	8	16	4	85
Total	7	—	4	5	15	24	43	31	20	13	20	5	187

Im Gegensatz zur Poliomyelitis und anderen durch Enteroviren bedingten Infektionen ist die FSME von Mensch zu Mensch nicht übertragbar. Die Durchseuchung der Bevölkerung nimmt daher nicht die gleichen Ausmaße wie nach Infektionen mit Enteroviren an und beträgt im Bezirk Neunkirchen auf Grund eigener serologischer Untersuchungen 14%.

Für die Übertragung der FSME auf den Menschen werden verantwortlich gemacht:

1. Der Kontakt, insbesondere der Stich infizierter Zecken,
2. der Genuß roher Milch von Tieren, die sich im Stadium der Viraemie befinden, und
3. möglicherweise auch der Kontakt mit infizierten Tieren bzw. deren Organen.

Um die Verhältnisse im Bezirk Neunkirchen näher aufzuklären, wurden im Lauf des Jahres 1959

1. von Juni bis September an verschiedenen Orten im Bezirk Neunkirchen 4672 Zecken aufgelesen. Dabei gelang es nur aus einem im Juni gefangenen Kollektiv von 900 Zecken (*Ixodes ricinus*) verschiedener Stadien, die als Pool von je 100 Larven bzw. 50 Nymphen auf empfängliche Mäuse übertragen wurden, zwei Stämme aus Larven und einen FSME-Stamm aus Nymphen, zum Teil erst nach der dritten Blindpassage, zu isolieren.
2. an verschiedenen Orten wilde Mäuse eingefangen, um aus den Gehirnen das FSME-Virus zu isolieren; dies gelang viermal aus *Microtus arvalis* und *Apodemus silvaticus*.
3. die Durchseuchung mit FSME-Virus jener milchproduzierenden Haustiere bestimmt, die im Haushalt von Patienten mit FSME standen.

	Neutralisierende Antikörper	Keine Antikörper
Rinder	11	22
Ziegen	2	4
Schafe	0	2

Isolierungsversuche aus der Milch von 24 Kühen und 4 Ziegen verliefen dagegen negativ.

4. alle Patienten mit FSME hinsichtlich Zeckenstich und Genuß roher Milch befragt.

Von den 13 Patienten gaben während der fraglichen Inkubationszeit zu:

4 von Zecken gestochen
5 Genuß roher Kuhmilch
1 Genuß roher Kuhmilch und Ziegenmilch.

Vergleichsweise wurden vom 15. 4.—31. 7. 1959 alle 792 neu aufgenommenen Patienten des Krankenhauses Neunkirchen in gleicher Weise befragt. Davon gaben zu:

16 von Zecken gestochen
117 Genuß roher Kuhmilch
und 11 Genuß roher Kuhmilch und Ziegenmilch.

Auf Grund der Untersuchungen und Erhebungen erscheint es glaubhaft, daß von den 13 Patienten mit FSME wie folgt infiziert worden sind:

4 durch Zeckenstich (Genuß roher Milch negiert)
4 alimentär (Zeckenstich negiert)
5 unbekannt.

Wenn man von den 8 vermuteten gefundenen Mechanismen der Übertragung absieht, so bleibt es doch auffallend, daß bei einem Teil der Patienten ein Kontakt mit infizierten Vertebraten oder Arthropoden nicht nachweisbar ist.

Angaben aus der Anamnese

Jahr	FSME-Fälle	Zecken			rohe Kuhmilch		rohe Ziegenmilch	
		ja	?	nein	ja	nein	ja	nein
1956	44	18	8	18	nicht durchgeführt			
1957	31	8	8	15	nicht durchgeführt		8	23
1958	14	4	2	8	3	11	2	12
1959	13	3	1	9	5	8	1	12

Dies geht auch aus den Angaben der Patienten in den vergangenen Jahren hervor, so daß man heute wohl zu Recht schon annehmen darf, daß zumindest hier in Niederösterreich die FSME auf den Menschen nicht durch Zeckenstich allein bzw. durch Genuß roher Milch frisch infizierter Tiere übertragen wird. Dafür spricht auch die Beobachtung, daß einzelne Krankheitsfälle in Monaten auftreten, in welchen ein Zeckenbefall nicht ortsüblich ist. Einige Hinweise, wie z. B. aktive Teilnahme an der Schlachtung von Haustieren in der fraglichen Inkubationszeit, scheinen dafür zu sprechen, daß man auch diesen direkten Infektionsweg ohne Mitwirkung von Zecken in Betracht ziehen muß. Inwieweit man noch mit anderen Arthropoden als Vektoren zu rechnen hat (Milben, Stechmücken usw.), ist noch nicht geklärt.

Die Zecken sind somit auch hier in Mitteleuropa von großer Bedeutung für die Verbreitung des Virus unter wilden, aber auch domestizierten Tieren sowie für einen Teil der infizierten Menschen; sie können aber nicht mehr als einziger Überträger des FSME-Virus auf den Menschen angesehen werden.

EXPERIMENTELLE UNTERSUCHUNGEN ÜBER DIE ÜBERTRAGUNG DES VIRUS DER FRÜHSOMMER-MENINGO-ENCEPHALITIS (FSME) IN DER ZECKE IXODES RICINUS

J. LOEW

Manuskript nicht eingelangt

ABSTRACT

Im Laboratorium gezüchtete virusfreie Zecken aller drei Stadien von *Ixodes ricinus* wurden zur Blutmahlzeit an Mäusen angesetzt, die 24 Stunden vorher mit FSME-Virus subcutan infiziert worden waren. Die infizierten Zecken wurden über einen längeren Zeitraum hinaus beobachtet, wobei folgende Befunde erhoben werden konnten: Der Virusnachweis gelang: 1. aus den Fäzes von Larven und Nymphen vor Beginn der Metamorphose, 2. bei Larven und Nymphen nach dem Saugakt bis zum Eintritt der Metamorphose, nicht während der

eigentlichen Umwandlung, jedoch später im nächsten Entwicklungsstadium bis 2½ Monate nach dem Schlüpfen, 3. bei Nymphen im folgenden Frühjahr, nachdem sie als vollgesogene Larven der natürlichen Winterruhe ausgesetzt worden waren.

Bei der Zucht der virusinfizierten Entwicklungsstadien im Laboratorium konnte kein Unterschied in bezug auf das physiologische Verhalten der Tiere im Vergleich mit den nicht infizierten Tieren der Zucht festgestellt werden.

DISCUSSION

J. A. CAMPBELL: Has Dr. Loew any information on the seasonal variations in tick activity in Austria? In Britain we have spring active ticks (all instars) and autum-active ticks (all instars) in the first of which diapause does not normally occur, although it appears when they are continously laboratory-bread. In the second diapause is invariable. These ticks thus overwinter in the engorged state and do not moult until the following year, whereas spring ticks moult in the summer of the year in which they lugaged and overwinter in the flat state. I do not find that light has the same role with British ticks in the induction and breaking of diapause such as Dr. Loew describes for Austrian material. Diapause incidence with us is closely related to the temperature pattern of the environment and can be induced and broken by temperature changes in total darkness.

SYMPOSIUM VII

ARTHROPODS IN RELATION TO BLOODPARASITES, ESPECIALLY THOSE OF WILD ANIMALS

SIMULIIDS, CERATOPOGONIDS AND HIPPOBOSCIDS IN RELATION TO BLOOD PARASITES OF BIRDS

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Our researches on avian haematozoa and their modes of transmission have provided opportunities to observe the feeding habits of several simuliids and biting midges and to assess their importance as intermediate hosts. Data will be presented to show that several species of these Diptera are ornithophilic and suitable hosts for one or more types of parasites occurring in the blood of birds. Moreover, some of these Diptera feed on some birds more than on others and in certain habitats; such feeding behaviour is most favourable for the transmission of particular parasites.

The work was done in Alonquin Park, Canada, where five types of parasites, namely *Plasmodium*, *Leucocytozoon*, *Trypanosoma*, *Haemoproteus* and filarioid nematodes, occur in birds. Species of *Plasmodium*, presumably carried by mosquitoes, are scarce in this locality and will not be considered further this afternoon.

Methods used to study the other four types of organisms in relation to the Diptera that feed on the birds included: (1) Examination of blood of miscellaneous birds. (2) Collection of flies from various birds at different times and in different habitats. (3) Exposure of birds to natural infections at various periods throughout the summer and in different habitats, to determine when they became infected. (4) Collection of flies that fed on infected birds, maintaining the flies in captivity, and at intervals thereafter examining them for developing stages of the parasites. (5) Injection of birds with stages of parasites found in the flies, to see if infections could be produced.

Birds were obtained for examination by various means but especially in Japanese mist nets. Plastic bags were placed over the birds before removal from the nets in order to collect hippoboscids that might be on them. Simuliids and ceratopogonids were collected from birds exposed on the shore of a lake, on the ground in a mixed boreal forest, and in the forest canopy 15 feet from the ground. Birds were confined in small cages as described by Bennett (6). After twenty minutes fine mesh cages were placed over these and left for 20 to 30 minutes. Engorged flies flew off the birds and were collected in an aspirator through sleeves in the tops of the cages. Birds were raised and lowered in the forest canopy by a rope and pulley arrangement.

The survey of 3000 birds of 84 species revealed the following incidence of blood parasites: *Leucocytozoon* 32%, *Trypanosoma* 18%, *Haemoproteus* 13%, filarioids 3%. Parasites occurred in almost 100% of some birds but were never observed in others. For example, most robins (*Turdus migratorius*) harboured *Leucocytozoon* but not *Haemoproteus*, whereas *Haemoproteus* was common in woodpeckers (Picidae) but *Leucocytozoon* was rare.

Following the early descriptions of avian haematozoa by Danilewsky (11), and others (9, 17—22), these and other species have been noted by several authors in a various birds throughout the world but details of the life cycles and transmission have been described for few. Skidmore (26) and O'Roke (24) reported simuliids as hosts for species of *Leucocytozoon*. Work in our laboratory has shown that ornithophilic simuliids are hosts for species of this parasite occurring in ducks and other birds (13, 15). Adie (1), O'Roke (23), Tarshis (27) and Baker (4) reported hippoboscids as hosts for *Haemoproteus*. We have shown that *Culicoides* spp. are hosts for at least two species and have data on others (14, 16). Mode of spread of avian trypanosomes is not clearly established although Baker (3) obtained transmission using hippoboscids. Transmission of most avian filarioids is still unknown, although Anderson (2) demonstrated simuliids as hosts for one species.

To obtain evidence of the kinds of insects that may serve as vectors of these parasites the incidence of infection with the various organisms and heights of parasitaemias in birds were noted throughout the summer and compared with the number and kinds of flies feeding on some of these birds (5). The comparisons indicated clearly: (1) Many immature birds were infected, therefore, vectors were prevalent in the locality. (2) The highest incidence and most intense infections with *Leucocytozoon* were noted at the time most black flies were captured after feeding on selected birds. (3) Trypanosomes were found in most birds when black flies and biting midges were present and before hippoboscids were taken. (4) *Haemoproteus* was present in juvenile birds several weeks before hippoboscids were taken and birds with intense infections were most numerous when black flies and biting midges were most abundant.

It was noticed also that 14% of 1750 infected birds harboured *Leucocytozoon* and *Trypanosoma* whereas less than 2% of those with *Haemoproteus* were infected also with trypanosomes. The reverse would be expected if hippoboscids were the vectors of *Trypanosoma* and *Haemoproteus*. The results suggest that simuliids and/or biting midges, rather than hippoboscids, are the important hosts of these three types of parasites in Algonquin Park. This view is supported by information obtained from the collections of simuliids and biting midges that fed on various birds when placed on the lake shore and in the forest and from the examinations of those flies that fed on infected birds. Noticeable differences were found in the number and kinds of flies collected. *S. rugglesi*, *S. euryadminiculum* and two new species were taken most often on ducks and at the lake shore. Most *S. aureum*, *latipes*, *Prosimulium decemarticulatum*, *Cnephia "U"*, were taken from the woodland types of birds when these were exposed in the forest rather than at the lake shore and in the forest canopy rather than on the forest floor (6). Similar differences in the feeding habits of ornithophilic *Culicoides* were noted. *Culicoides* near *piliferus* was taken most abundantly from ducks on the lake shore and after dark. *C. crepuscularis*, *stilobezzioides*, *sphagnumensis* and *haematopotus* were recovered in largest numbers from woodland birds when exposed in the forest canopy. Clearly these midges and simuliids, as well as others, fewer of which were taken, are ornithophilic and, if hosts of avian parasites, should be most satisfactory vectors. Hippoboscids, as already pointed out, were not taken until early July and even then in relatively small numbers.

The life cycles of particular species of these various parasites were investigated, therefore, to determine which flies are suitable intermediate hosts. *Leucocytozoon simondi*, which was thought to be transmitted to ducks by the black fly *Simulium venustum* (12, 24), has received most attention. During several seasons observations were made on ducks to ascertain when they became infected naturally. These observations, together with collections and examinations of flies feeding on infected ducks, have shown that ornithophilic flies rather than *S. venustum* feed commonly on ducks and serve as hosts for the parasite (13). Transmission begins some years in mid-May at which time *S. euryadmini-*

culum and two new species are feeding on ducks, whereas *S. rugglesi* feeds on them in June and July and is the important host at this time. A sequence of stages has been found in *S. rugglesi* and infections have been produced by injecting the final stage—the sporozoite—into other ducks. Species of *Leucocytozoon* in grackles (*Quiscalus versicolor*) white-throated sparrows (*Zonotrichia albicollis*), ruffed grouse (*Bonasa umbellus*), robins (*Turdus migratorius*), saw-whet owl (*Aegolius acadica*), and blue jay (*Cyanocitta cristata*) have been subjected to similar, although less complete, studies. Stages of the parasites have been found, and infections produced, by transferring sporozoites to other birds. Development of these several species of *Leucocytozoon* has been demonstrated in ornithophilic simuliids in the genera, *Simulium*, *Prosimulium* and *Cnephia*.

If suitable flies actually seek out and feed on the hosts that are susceptible to the parasite, eventually most of the flies should be carrying sporozoites. A measure of the extent to which this happens was obtained by noting the percentage of flies with sporozoites in a sample of woodland species taken weekly throughout the summer. More than 50% of several species were carrying sporozoites in July. A similar situation was found in a sample of *S. rugglesi*. Consequently, conditions are optimal for the transmission of parasites for the vectors feed almost exclusively on birds that can carry the parasites—a situation rather unique in parasitology.

All of these simuliids possess the basal tooth on the tarsal claw, a feature that Shewell (25) considers characteristic of ornithophilic species. It will be interesting to learn whether this applies to species elsewhere. Crosskey (10) has noted the tooth on *S. griseicolle* but points out that *S. damnosum* is an interesting exception, as it possesses the tooth but feeds on mammals including man.

Discovery of the intermediate hosts of species of *Haemoproteus* came as a sequel to work on *Leucocytozoon*. High incidence of infection with *Haemoproteus nettionis* in ducks, and failure to find hippoboscids on them led to the conviction that hippoboscids were not vectors. Biting midges were suspected when it was found that thousands of these belonging to a new species near *piliferus* fed on ducks after dark. Ducks, left out of doors at night only, became infected with *Haemoproteus* but not *Leucocytozoon*. Examination of specimens of *Culicoides* that fed on infected ducks revealed stages of the parasite. Sporozoites from these midges produced infections when they were transferred to ducks (14). The preliminary results of this work were available just in time to report briefly at the previous International Congress meeting in Montreal.

The investigation was extended to include species of *Haemoproteus* in other birds. Transmission of *H. canachites* by *C. sphagnumensis* has been established (16). Moreover, sporozoites of *Haemoproteus* from other birds have been demonstrated in the salivary glands of *C. stilobezzioides* and *C. crepuscularis* indicating their suitability as hosts. Further support for the conclusion that *Culicoides* rather than Hippoboscidae are hosts for *Haemoproteus* in our locality comes from observations on Hippoboscidae by Bennett (8). Infections with *Haemoproteus* did not develop in birds on which hippoboscids (*Ornithomyia fringillina*) were placed which had fed previously for one to two weeks on infected birds. Moreover, the hippoboscids did not shift readily from one bird to another—an essential requirement if they are important vectors. In addition, *Haemoproteus* is common in ducks and in purple finches (*Carpodacus purpureus*) but hippoboscids do not occur on the former and live for a short time only on purple finches.

Investigations of avian trypanosomes by Bennett (7) indicate that ornithophilic simuliids are suitable hosts for trypanosomes occurring in nine species of birds that have been tested thus far. Some trypanosomes developed to the infective stage also in *Culicoides*, *Ornithomyia fringillina*, and in certain mosquitoes. However his evidence points to simuliids, rather than these other Diptera, as the important vectors.

In so far as filarioids are concerned, we know that development of species other than

that reported by Anderson, occurs in *Cnephia "U"*, *Prosimulium decemarticulatum* and *Simulium aureum*, *S. latipes* and *S. croxtoni*.

These various observations indicate that species of *Leucocytozoon*, *Trypanosoma* and filarioid nematodes are transmitted by ornithophilic simuliids and *Haemoproteus* by species of *Culicoides*. Transmission of some of the parasites is especially favoured by the feeding behaviour of the flies.

Finally, it might be noted that ornithophilic simuliids and ceratopogonids do not appear to feed on man. However, species that feed on mammals, including man, feed occasionally on birds. Consequently, circumstances can be visualized in which ornithophilic flies could maintain a parasite in nature in wild birds from which only sporadic transfer to mammals would occur if mammalian feeding flies fed on an infected bird and then on a mammal. With the current interest in the transfer of viruses from birds to man perhaps the speculation should not be overlooked.

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THE PERITROPHIC MEMBRANE OF BLOOD SUCKING DIPTERA IN RELATION TO THEIR ROLE AS VECTORS OF BLOOD PARASITES

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Usually the peritrophic membrane (PM) of insects is only known and described morphologically as a part of the midgut and its presence and function is not clearly understood (Day & Waterhouse 1953, Wigglesworth 1953). It is generally agreed that the PM protects the cells of the midgut from hard or sharp particles of food, though in many insects which suck blood or fluids of plant origin, a PM can be found. The PM consisting mainly of chitin is readily permeable to digestive enzymes and products of digestion, and a number of dye-feeding experiments suggest that the membrane acts as an ultra-filter. Thus the PM forms a barrier permeable only to extremely small particles. Three different types of PM are found in insects and can be characterized as follows:

Type I of the membrane consists of a series of thin concentric lamellae, independent or loosely attached to one another. The formation of these lamellae is not quite clear but it is thought that they are produced periodically by the separation of thin sheets from the surface of the cells throughout the length of the midgut.

The second type of the membrane consists of a single uniform layer. It is secreted continuously in viscous form by a group of cells at the anterior end of the midgut. It soon becomes solid and is of uniform circumference throughout its length. Both these types are produced irrespective of the intake of food.

The third type of membrane is formed only after a meal (Stohler 1957). The material is secreted in viscous form by a part of or the whole midgut epithelium. It stiffens, completely enclosing the gut content and is excreted at the end of digestion.

After this introduction we come to the point of this survey. Under discussion is the way parasites, transmitted by blood sucking dipteras deal with the PM, which evidently forms a barrier in their way from the lumen to the gutwall and other parts of the insect's body. An answer to the following questions will be attempted:

1. Is there a PM in important vectors of insectborne diseases?
2. Does the PM form any barrier or obstacle for parasites? and if so:
3. Can parasites readily penetrate the PM or are there ways to by-pass the membrane?

To begin with we take the example of the different trypanosome cycles in the tsetse fly (Geigy & Herbig 1955). These cycles show an increasing adaptation to the morphologic conditions in the insect gut, where a PM of type II is found. The cycle of *Trypanosoma vivax* is the simplest as only trypanosomes which can fix themselves in the hypopharynx are transmitted, while those taken into the gut are digested. But already with *T. grayi* the PM of the Glossina has a certain effect. While the blood is digested in the endoperitrophic space, the trypanosomes pass to the end of the PM, penetrate the ectoperitrophic space where they finish their evolution and finally come to rest in the hindgut still outside the PM. From there the metacyclic forms are excreted little by little with the rest of the digested blood. A further complication is found in the case of *T. congolense*. As before the end of the membrane is by-passed and the trypanosomes travel in the ectoperitrophic space to reach the region where the PM is formed near the proventriculus. Here the membrane is still soft and can be penetrated actively by

the trypanosomes. Finally they reach the hypopharynx from where they are transmitted to another host on the occasion of a blood meal. In the case of the trypanosomes of the brucei-group, i.e. *T. rhodesiense*, *gambiense*, and *brucei*, the route is the same as before but prior to reaching the infective stage, they must pass to the salivary gland from where they are injected into a new host.

Summarizing the facts we can say that the PM of tsetse flies cannot be penetrated with the exception of a short region at the place of formation, however trypanosomes find opportunities enough to by-pass the membrane at its open end.

Another example of interactions between membrane and parasites is found in the case of phlebotomes which act as vectors for Leishmaniasis. Here we find a PM of type III, i.e. after a blood meal a membrane forms around the whole content of the midgut. In this way the parasites are enclosed in the peritrophic sac. In three different species of phlebotomes what happens next has been studied (Feng 1951). In *Phlebotomus mongolensis* the PM forms a complete sac throughout its existence, the flagellates enveloped within. As the digestion of the blood goes on, the flagellates decrease in number. At the end the peritrophic sac is discharged together with the remains of the blood and the flies become free of infection. In contrast to this the PM in *P. chinensis* also forms a sac, but later it breaks especially at its posterior end and the flagellates are set free in the midgut, they invade the proventriculus in which they become established. Finally in *P. squamirostris*, vector of the toad trypanosome *T. bocagei*, the PM also forms a sac initially. But in this case it is not tightly closed, so that after some time portions of the digested blood are discharged and flagellates begin to pass down the end gut, they attach themselves to the gutwall, multiply and develop into the infective stage.

No corresponding studies have been made with other species of sandflies. Anyway from these results we can conclude that the PM of sandflies cannot be penetrated by parasites, at least when the membrane has become solid. Later the condition of the membrane decides further progress of an infection.

Also simuliids possess a PM of type III. Its role in parasitic infections was studied in *Simulium damnosum* infected with *Onchocerca volvulus* (Lewis 1950 & 1953). The PM formed at the time of the blood meal envelops the content of the midgut and remains unbroken for at least 24 hours. The average number of microfilariae ingested was usually found to be considerably greater than that of larvae completing the developement. Subsequently most of the ingested microfilariae were seen imprisoned in the peritrophic sac. Only few remain in the tubular part of the midgut where no membrane is formed and it is thought that only these few can make their way to the thoracic muscles. Further it is possible that some of the microfilariae can escape from the PM during its viscous phase soon after the formation. But still the majority must be too late to penetrate. Only exceptionally in very heavily infected flies does it appear that the numerous microfilariae can prevent the formation of a proper membrane. In this case the flies become overinfected and die.

From these facts it is possible to conclude that, once formed, the PM of *Simulium damnosum* is normally unpenetrable for enclosed microfilariae and thus protects the fly from heavy infections without preventing transmission of the disease.

The last example of relations between parasites and PM in dipteras is found in mosquitoes. Experimental studies were made with *Aedes aegypti* and the parasite of chicken malaria *Plasmodium gallinaceum* (Stohler 1957). The PM of Aedes is also secreted in fluid form following each blood meal. In the course of 20—30 hours it grows more and more solid. Up to the 30th hour after the infective meal ookinetes readily penetrate the membrane and reach the cells of the midgut. The rest finds themselves captured in the

peritrophic sac and die, usually lining the now solid membrane. Therefore it seems that as in the case of Simulium the PM of Aedes has a regulative function on the infection with the malaria parasite.

At the end a rather speculative idea about the influence of the PM on infections must be mentioned. It was found that it was impossible to infect certain species of mosquitoes with viruses of Murray Valley encephalitis and western and eastern equine encephalitis when the mosquitoes were fed on infected animals. But when suspensions of viruses were injected into the body cavity or when the gut filled with blood was perforated with a needle, infections took place and the mosquitoes were able to transmit the disease normally by bite (Hurlbut 1951, McLean 1953, 1955, Merrill & Tenbroeck 1935). These results could be easily explained supposing that in certain cases an intact PM would intercept a natural infection. Bearing in mind all cases where connections between PM and parasites have been found it seems permissible to summarize the results and to answer the questions put earlier as follows:

In most vectors of parasites which must fulfil part of a cycle in an insect a PM is found. It forms a real barrier and cannot be penetrated. But as was to have been expected from acknowledged vectors of parasites, membranes can be either by-passed at the open end (type II) or parasites force their way through the membrane just after its secretion in viscous form (type II and III). Eventually a solid membrane breaks and sets the enclosed parasites free. Therefore the PM does not prevent the infection of an insect, at best the degree of an infection is influenced.

On the other hand the possibility that the PM prevents a transmission can not be excluded, though it has not yet been found. In this respect one must think of numerous cases where related species of insects are either good, bad or no vectors, without any obvious reason for such behaviour being found. Possibly a study of the respective circumstances of the PM could bring some light to these cases.

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SOME ASPECTS OF THE BEHAVIOUR AND PHYSIOLOGY OF BITING FLIES THAT INFLUENCE THEIR ROLE AS VECTORS

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In this paper we discuss some of the behavioural and physiological factors that influence the insect vector in taking its meal of blood. The discussion will be restricted to the Culicidae, Simuliidae and Ceratopogonidae and the examples will be chosen mainly from North American studies with which we are familiar.

To be a vector the insect must take at least two blood meals. Typically the blood meal and ovarian maturation are intimately related and the ingested blood provides both the stimulus and the nutrition for the development of the oocytes (6, 17). The whole process from feeding to laying is spoken of as a gonotrophic cycle, and may require from two to perhaps 10 days according to species and conditions.

Very important in determining the capacity and efficiency of a vector are the host range and host preferences. They can be studied not only by observation and experiment but also by identifying, by serological means, the ingested blood (1, 11, 14, 16). These studies indicate that some species are restricted to a particular natural group, e.g., mammals, or birds, or cold-blooded vertebrates. Certain species and groups of black flies, and several species also of *Culicoides*, are characteristic of birds (3) and Shewell has pointed out that the bird-feeding black flies agree in having toothed claws (34). *Culicoides nubeculosus* (Meigen), *C. variipennis* (Coquillett) and their allies are associated chiefly with man and large domestic animals, and an undescribed species near *C. piliferus* has been taken only on turtles (Wirth, in litt.); but *C. obsoletus* (Meigen) and others feed on both mammals and birds. Perhaps feeding habits are rarely quite rigid; thus *Eusimulium latipes* (Meigen), which usually feeds on birds, will attack several mammals including man (11). There is little evidence that the host range is ever very narrowly limited; perhaps the species that feed on livestock (Ungulata), or the *Culicoides* on turtles, are as restricted as any.

Several stimuli may be involved when a biting fly lands on its host, among them being carbon dioxide, moisture and warmth (4), and perhaps also a more specific chemical from the blood (33). There is also a visual element, depending on the colour and brightness of the surface (4). The response to these factors, doubtless differing from species to species, will influence the conditions and efficiency of host finding and hence both the host range and the population level necessary for survival and for efficiency as a vector. Thus a response to colour is of no significance to a species active in full darkness; in practice this is probably rare but may be realized in *Culicoides austeni* Carter which feeds at night sometimes inside native huts (22). Similarly a response to warmth would be of no value to *Culex apicalis* Adams, which feeds on frogs. Reeves found that carbon dioxide released at different rates attracted mosquitoes of different food preferences, and that *Culex tarsalis* Coquillett, a species with many hosts both avian and mammalian, responded to all emission rates (1, 29). This wide range, it may be added, enables *C. tarsalis* to be an important vector of western equine encephalitis, but might well handicap it with respect to a more specific parasite.

Such factors however can attract only over the short range in which they produce a gradient sufficient for orientation. In tse-tse flies a long range visual response is known, but this has rarely been suggested in biting Nematocera. Nevertheless Knab

observed prairie species of *Aedes* flying towards conspicuous objects (27), and *Aedes hexodontus* Dyar flies upwind towards the observer from at least 30 metres. The latter is interpreted as a visually controlled movement that brings the insect close to the host and ultimately under the influence of landing gradients (19, 26).

The factors that control the rhythms of activity and function are also relevant. Thus black flies that attack birds feed at late dusk, after the manbiting species have disappeared, and often at a considerable height above ground. Presumably many birds can be attacked readily only when roosting (3). *Mansonia perturbans* (Walker), which flies near the ground by day and higher up at night, attacks both birds and mammals (15, 35).

Not infrequently, however, host selection is controlled more by availability than by true preference. Thus *Eusimulium latipes* can feed on many birds, but around farms it attacks chiefly *Gallus*; and *Aedes* spp. feed on large and small mammals in proportion to their surface area (11, 14).

The precipitin test sometimes shows that a fly contains blood derived from more than one kind of host. The technique is limited, and cannot detect multiple feeding on the same host nor feedings separated by too great an interval. The results show that certain species are more ready than others to interrupt and resume feeding (11, 15). This quality will depend on the level of response to factors that maintain ingestion, e.g. adenosine-5′-phosphate in the blood in the case of *Culex* (23), and on the behavioural reaction, also in part inherited and specific, to the adverse actions of the host. The resultant degree of "timidity" could influence the efficiency of a vector strongly, by increasing the number of animals attacked or by decreasing the chance of acquiring a minimum effective dose. Especially in purely mechanical transmission the frequency of multiple feeding could yield a useful index of efficiency.

The more painful the bite the greater the chance that the insect will be interrupted or harmed, and Herms (21) long ago suggested that efficient vectors are likely to be relatively benign biters. It is of great interest therefore that Hudson et al. (24) have recently shown that the saliva of mosquitoes contains a substance with anaesthetic properties.

Since the vector must survive to transmit the parasite—and in some cases, e.g. *Culicoides variipennis* and the virus of blue tongue, the infective stage is not reached until the time required for two gonotrophic cycles has elapsed (25)—all the factors that affect survival become relevant. Thus we must take account of weather, available water and energy sources (nectar), natural enemies, the danger of death on biting and many other elements. The "average life" of adult *Anopheles culicifacies* Giles has been given as four days (32), but such low estimates should be balanced by the studies of the ovariolar dilations that remain after oviposition, which show that some individuals in *A. maculipennis* at least survive to complete many gonotrophic cycles (13). In *Simulium ornatum* Mg. both female longevity and population size are greatest in late summer, leading to far higher numbers of potential vectors at that time than at any other (9). In virtually univoltine black flies, such as *S. venustum* Say, the pattern of dispersal in relation to population size could determine the rate of parasite transmission. Although high numbers of young flies may occur as far as one mile from a breeding site, by the time the population has aged sufficiently to consist largely of old flies, these exist in large numbers only close to the breeding stream, presumably because old flies disperse more slowly than young ones (10). The probability of infective bites thus decreases rapidly with distance from the stream, and the scale of the pattern would vary with the population size and the dispersal habits of the species.

The one to one relationship between feeding and maturation is not universal. *Anopheles gambiae* Giles, for instance, needs two meals to complete the first cycle (7); and *Anopheles*

spp. in gonotrophic dissociation, and *Aedes* spp. in old age (12), feed repeatedly without ripening eggs. There is also the phenomenon of autogeny, of widespread occurrence and including two grades of very different significance. Species such as *Prosimulium fuscum* Syme and Davies and *Culicoides gigas* Root and Hoffman begin adult life by ripening a group of eggs and only then seek a blood meal, with which the second cycle is initiated. As vectors they would tend to be inefficient, since the first cycle is of no account, unless, of course, they pass through numerous later cycles. In others, such as *Prosimulium ursinum* Edwards and an undescribed *Culicoides*, there is only one ovarian cycle; the insects do not attempt to bite and are often, because of structural reduction, unable to do so. One or both grades of autogeny occur not only among mosquitoes but also in several species groups in *Prosimulium*, *Cnephia*, etc., and in five sections of *Culicoides*; and frequently autogenous species are very closely related to normal forms (*P. mixtum* Syme and Davies and *C. variipennis*, for examples cited) (8, 10, 18, 20). Sometimes autogeny occurs in only a fraction of the population, as in *Culex pipiens* and *Aedes taeniorhynchus* (Wied.), and the fraction varies from place to place (28, 30). It is known in *Culex tarsalis* (2, 5), an important vector species, but the natural frequency has not yet been established. In black flies Rubtzov finds that it is determined environmentally (31).

Autogeny is of adaptive value if finding the vertebrate host is dangerous or uncertain, and it is perhaps no accident that most of the autogenous species of *Culicoides* and Simuliidae live in open windswept environments, the sea coast, the prairie and above all the arctic tundra (20, and later observations).

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THE PROBLEM OF RESERVOIRS AND THE ECOLOGY OF PHLEBOTOMUS

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Manuskript nicht eingelangt

ABSTRACT

American muco-cutaneous leishmaniasis (*Leishmania brasiliensis* and its geographical races) is widely distributed in the tropical forests of the New World. All epidemiological evidence points to some animal other than man as the reservoir. Natural infections of Leishmania, demonstrated by culture of heart blood drawn from the living animal, have been found in two genera of spiny rats, Proechimys and Hoplomys. Over thirty other genera of wild-caught forest animals have been negative. Tested in a volunteer, one spiny rat strain produced a typical human lesion. Panamanian strains of whatever origin, animal or human, in culture or in biopsy tissue, inoculated into a variety of animals have so fair failed to produce infection, with the sole exception suckling white mice, in which intradermal inoculation produces a transient, localized infection of the cells of skin connective tissue. *Phlebotomus* sandflies which freely attack amn, horses and other large animals, are abundant in all endemic foci, the principal species being *trapidoi*, *sanguinarius*, *panamensis*, *gomezi*, *ylephiletor* and *paraensis*. In work on the ecology, of which little was previously known, particular attention has been paid to the search for breeding and resting places. By a method of screen-washing and flotation in saturated sugar solution, about 2500 larvae have been recovered, of which several hundred have been reared to the adult stage and identified (at least twelve species). There is a sharp division between the man-bitting species and other forest sandflies as to habitats used as breeding places and diurnal shelters. The adults of a group of about ten species which do not attack man are characteristically found in tree buttresses, hollow trees and animal burrows. The larvae recovered from soil in such habitats belong to this same group. The man-biting species, with one exception, are almost never found in such places but occur diffusely scattered over the forest floor in fallen leaves or resting on the under sides of green leaves. The larvae of two members of this group, *panamensis* and *paraensis*, have been found in layers of rotting leaves, not in soil, and it is likely that larvae of the other man-biting species will also be found scattered over the forest floor. There is in progress the determination of what animals other than man are attacked in nature, by means of precipitin tests and other methods. To infect sandflies for transmission experiments, it has been necessary to resort to artificial feeding by the Hertig pipette technique. In all of the five species tested typical flagellate infections have been produced in an average proportion of over 75 percent. The refeeding of known positive sandflies on spiny rats has so far resulted negatively. In the current phase of these transmission experiments human volunteers are being used.

RECENT OBSERVATIONS ON THE ECOLOGY OF THE SIMULIUM VECTORS OF ONCHOCERCIASIS IN UGANDA

G. R. BARNLEY

Manuskript nicht eingelangt

ABSTRACT

McMahon has described the phoretic association between the pre-adult stages of *Simulium neavei* (Roubaud) and the fresh water crab *Potomonautes niloticus*—*Potamonautes* (*Acanthothelphusa*) *niloticus* of R. Bott—and in the areas of Kenya from which he has eradicated *S. neavei* he found that the man-biting type-form associated only with this single species of crab which has a range conveniently limited to the more open and unshaded stretches of the larger and more rapidly flowing rivers. In Uganda the author has shown that there is no area drained by free flowing streams as apposed to papyrus choked rivers which is free from *S. neavei* transmitting Onchocerciasis. At least two other species of crab, *Potamonautes* (*Lirrangopotamonautes*) *johnstoni johnstoni* and *Potamonautes* (*Rotundopotamonautes*) *beradi beradi* and possibly a third, *P.* (*Rotundopotamonautes*) *granviki* are involved in phoretic association with *S. neavei* in Uganda. The habits of these crabs in relation to the pre-adult life of the associated *Simulium* and as complication factors in relation to *Simulium* distribution and control, are discussed. The aestivation of fertilised female *S. damnosum* along dried out river beds under dry season conditions is discussed as a factor complicating operations for the larvicidal control of this species.

HAEMOLAELAPS AEGYPTIUS KEEGAN, 1956 AS A VECTOR OF HEPATOZOON BALFOURI (Laveran, 1905)

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Introduction

Owing to the exceptionally wide diversity of insects, mites, and ticks that transmit some 50 species of *Hepatozoon* to an equally widely differentiated variety of vertebrate hosts in many parts of the world, one might question whether the specificity of arthropod-protozoon relationships in this assemblage is as narrow as it is among most other blood-infecting protozoa. Indeed, a single species, *H. pitymysi*, is transmitted by two species in different genera of fleas and by one species of Anoplura.

One *Hepatozoon* species easily available to us for research is *H. balfouri* (Laveran, 1905), which infests jerboas (*Jaculus* spp.) in ecologically and faunistically distinct desert and semidesert areas of Sudan and norther Africa, from Egypt to Tunisia. No vector of this infection was previously known.

In Egypt, populations of two jerboa species, *Jaculus j. jaculus* of desert and desert edge biota, and *J. o orientalis* of coastal semidesert biota (Fig. 1), have mean rates of *H. balfouri* infection of 41 % and 21 %, respectively. In view of the factors noted above—variety of vectors, ecological zones, faunal areas, and vertebrate hosts—the experiments described below were designed not only to discover a single vector of *H. balfouri* in Egyptian jerboa populations, but also to determine whether two or more kinds of arthropods may play a role in transmission of this organism to these two rodent species in their different ecological habitats.

Material and Methods

Approximately 1500 jerboas of both species were dug from their nests, trapped, or netted at night during each season and in most ecological zones and geographical areas of Egypt where they occur. Blood samples were taken from each animal and ectoparasites, e.g. lice (*Anoplura*), mites, and fleas, from all infected hosts were examined, by smearing or sectioning, for sporogonic stages of *H. balfouri*. Several hundred nests of jerboas were dug from burrows and parasites from them were studied by the same techniques. All arthropod species normally infesting Egyptian jerboas and several of rare or infrequent parasites were examined.

In addition, most common species of mites, fleas, and ticks parasitizing Egyptian *Jaculus* were cultured in the laboratory. Some were reared on infected animals and selected at random for examination. Others were reared on non-infected jerboas, fed experimentally on infected jerboas, and examined by smears or sections from two hours to two weeks afterwards for signs of infection with *H. balfouri*. Besides those mites normally found on local jerboas, cultures of three other easily reared species were established for experimental feeding on infected animals.

Results

Table 1 shows the kinds and numbers of ectoparasites removed from jerboas naturally infected with *Hepatozoon balfouri*. All specimens appeared to be negative for sporogonic stages of this organism. Included in this list are all ectoparasite species common to either or both species of *Jaculus* in Egypt. A few other arthropods are of such unusual occurrence on these animals or in their nests that they scarcely need to be considered in the epidemiology of *H. balfouri*.

Table 2 shows the kinds and numbers of ectoparasites that were reared in the laboratory. This list includes a number of the ectoparasites commonly infesting *Jaculus* species in Egypt. Several other arthropods infesting these animals could not be reared in the laboratory, e.g. *Eulinognathus aculeatus*. Also included among these experimental arthropods were some parasites, such as ticks, that uncommonly attack jerboas but were considered to be worth some investigative effort. No developmental stages of *H. balfouri* were found in any of these ectoparasites with the exception of certain cultures of the mite *Haemolaelaps aegyptius* to be discusses below.

Table 1

Ectoparasites removed from Egyptian Jerboas infected with *Hepatozoon balfouri* and examined for sporogonic stages

Species	Number Examined
FLEAS	
Mesopsylla tushkan subsp. nov.	46
Synosternus cleopatrae	2
Stenoponia tripectinata acmaea	18
Xenopsylla cheopis	44
Xenopsylla nubica	73
Species unidentified	37
LICE (ANOPLURA)	
Eulinognathus aculeatus	118
MITES	
Species unidentified	288
Androlaelaps marshalli	386
Eulaelaps stabularis	17
Haemolaelaps insulptus	116
Haemolaelaps aegyptius	475
TOTAL	1620

Table 2

Laboratory reared ectoparasites fed on *Hepatozoon balfouri*-infected jerboas and examined for sporogonic stages

Species	Number Examined
NORMAL PARASITES OF EGYPTIAN JERBOAS	
FLEAS	
Xenopsylla cheopis	34
Xenopsylla nubica	62
Mesopsylla tushkan subsp. nov.	70
Stenoponia tripectinata acmaea	213
MITES	
Androlaelaps marshalli	113
Haemolaelaps aegyptius	± 2000
TICKS	
Rhipicephalus s. sanguineus	
Larvae	± 476
nymphs	± 60
Hyalomma impeltatum & *H. dromedarii*	
Larvae	17
nymphs	42
Ornithodoros erraticus	± 40
SPECIES NOT OCCURRING IN NATURE ON EGYPTIAN JERBOAS	
MITES (over 2000 specimens)	
Haemolaelaps inops zulu	
Echinolaelaps echidninus	
Ornithonyssus bacoti	

Non-obligate parasites, such as mosquitoes, sandflies, hemipterous insects, etc., are absent or so rare in association with Egyptian jerboas that it is unlikely that they play a role in the epidemiology of *H. balfouri* in Egypt. Attempts to feed two species of

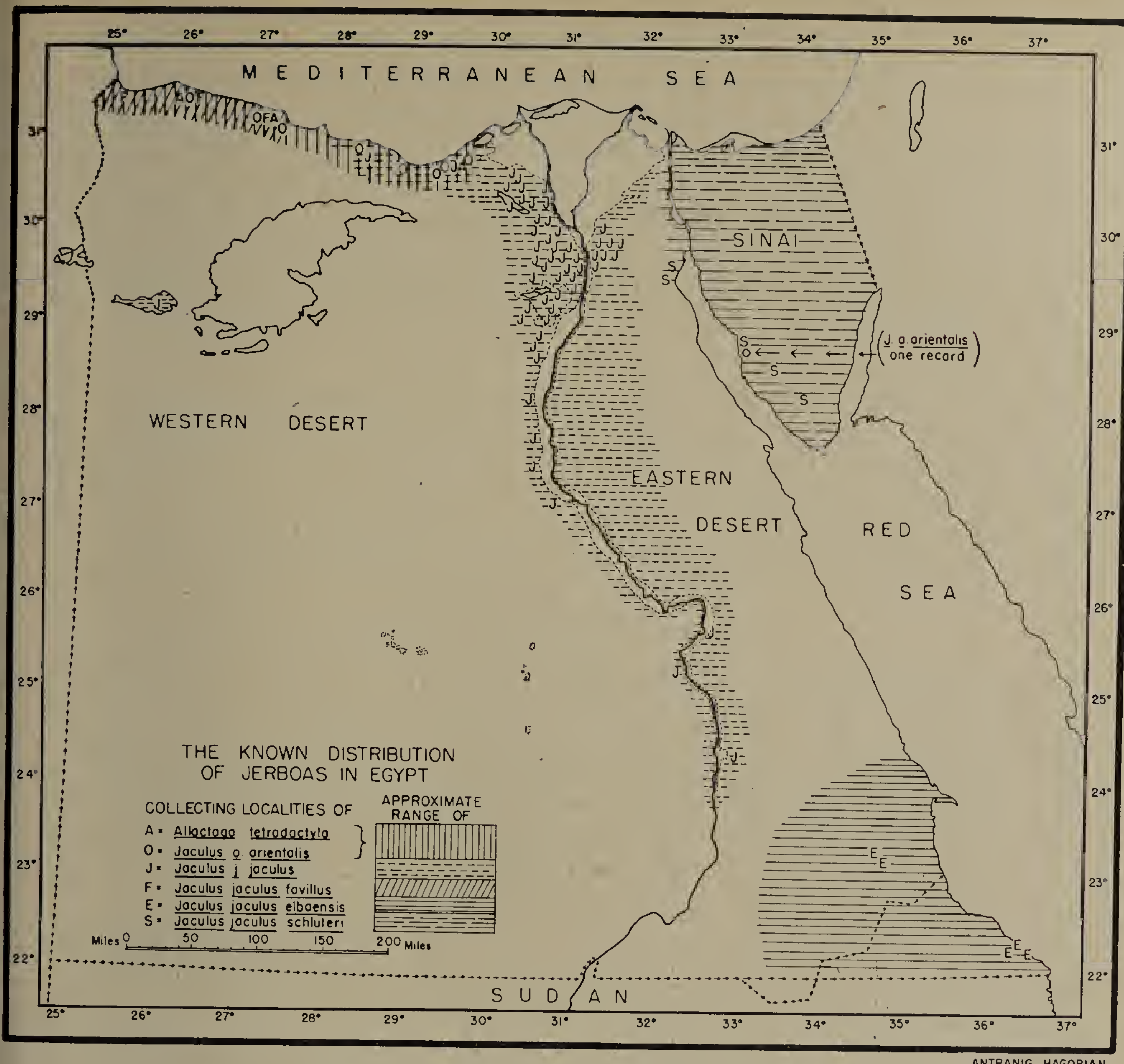

Figure 1. Distribution of Egyptian jerboas.

mosquitoes (*Anopheles pharoensis* and *Culex pipiens*) and one of sandflies (*Phlebotomus papatasi*), on infected jerboas were unsuccessful.

Haemolaelaps aegyptius, a common parasite of both *Jaculus* species in Egypt, could be infected experimentally under certain conditions. However, approximately 475 adult mites of this species removed in the field from over 150 naturally infected jerboas showed no sign of protozoal infection.

H. aegyptius colonies from 8 localities were established in the laboratory, using either infected or uninfected jerboas as hosts. When uninfected animals were utilized, samples of 20 to 80 female mites were starved for several days, then fed on infected animals, and examined from a few hours to 12 days afterwards. Samples from colonies reared on infected animals were taken at random after the colonies had become well established. Mites from only one of these 8 localities became infected. In this single area, El Qatta, *Jaculus j. jaculus* populations have a significantly higher rate of infection than have those of any other jerboas, either *J. j. jaculus* or *J. o. orientalis*, examined during this study.

The first three experiments with El Qatta populations of *Haemolaelaps aegyptius* were performed as follows:

A large culture of mites (number 309) was divided into three separate parts. Two of these lots, consisting of approximately 90 females and a number of males, were starved for 10 days; then each was placed on a separate infected jerboa for several hours of feeding, removed, and placed in a bottle (at 28° C) containing a baby mouse for additional food. A large proportion of the female mites in both samples showed sporogonic stages typical of *Hepatozoon* species when examined between 24 hours and 12 days afterwards. The third sample was handled as above except that after feeding on an infected jerboa and being placed in a bottle, the mites were offered a baby mouse for additional food only on the subsequent fourth and eighth days. Mites removed from this third lot and examined 6, 10, and 12 days after feeding on an infected jerboa were all negative for *H. balfouri*.

As a result of this experience, which appeared to suggest that a constant source of food might be necessary before sporogony of *H. balfouri* could proceed in *Haemolaelaps aegyptius*, further colonies of this mite, all from El Qatta, were developed on infected jerboas and samples were removed for examination when the number of mites became sufficiently dense. In all of these, the rate of *H. balfouri* infection was very high.

Discussion and conclusions

Haemolaelaps aegyptius Keegan, 1956, as now recognized, is one of the more common and versatile mites of Egyptian feral rodents. It occurs on and in nests of gerbils of various genera, *Gerbillus* spp., *Meriones* spp., and *Psammomys o. obesus*, as well as on jerboas, *Jaculus jaculus* subspp. and *J. o. orientalis*, and occasionally on *Rattus* spp. It is found in desert and desert edge zones in Sinai and the Eastern and Western Desert, for the entire length of the Nile Valley and Delta of Egypt, as well as in remote desert areas and oases. Significantly for the present study, this species has not yet been found outside of Egypt.

Results of experiments with mite culture 309 (*Haemolaelaps aegyptius*) suggest that if the sporogonic cycle is to proceed in these arthropods further blood meals must be available continuously for several days after ingestion of *Hepatozoon balfouri* gametocytes.

However, a large number of this and other species of mites removed from naturally infected jerboas in the field or reared on infected jerboas in the laboratory were negative for *H. balfouri* except for cultured mites originating from the single source, El Qatta. It appears fairly obvious that *H. aegyptius* is probably the chief vector in the El Qatta area. Populations of the same species of mite, taxonomically undifferentiated by presently known criteria, infest both *Jaculus* species almost wherever they occur in Egypt. Possibly, "strains" of this mite in other areas are less susceptible to infection than is that infesting El Qatta jerboas.

When these results were obtained, the problem was referred to Dr. Conrad Yunker, who thereupon undertook an exhaustive taxonomic study of large series of *H. aegyptius* from all hosts and areas where it is known to occur. Dr. Yunker subsequently reported that two or more subspecies might be represented in *H. aegyptius* populations, but that critical criteria remain unclear and in need of further study. Thus we have a taxonomic-physiologic question to be answered by morphological or biological studies, or both, before we can evaluate the role of *H. aegyptius* as a vector throughout its entire geographic range.

We might have been satisfied, after the large volume of examination of numerous jerboa-infesting arthropods described above, to consider experimental infection of only *H. aegyptius* as clear-cut evidence that this mite is the vector in Egypt and all other local arthropods can be disregarded. This assumption, however, appears to be

unsafe. Entirely negative findings of infection in *H. aegyptius* specimens removed from naturally infected hosts, as well as differences in ability to produce infections in this mite experimentally, indicate that a number of epidemiological factors in relation to this species as well as to all others parasitizing jerboas remain to be studied. Probably all jerboa ectoparasites require further investigation before their individual role in *H. balfouri* epidemiology can be evaluated. In summary of presently available evidence, it can be stated that certain populations of *Haemolaelaps aegyptius*, a common parasite of Egyptian jerboas, are infective with *Hepatozoon balfouri* but that the relative importance of *H. aegyptius* in the transmission of this infection elsewhere is uncertain.

THE BEHAVIOUR OF WUCHERERIA IN THE MOSQUITO HOST

B. R. LAURENCE

The microfilariae produced by adult filarial worms are usually found in the peripheral blood of the vertebrate host and may then be taken up by the arthropod host during a blood meal. *Wuchereria malayi* Brug is a parasite of man and both wild and domestic animals, and the related species, *W. pahangi* Buckley & Edeson and *W. patei* Buckley, Nelson & Heisch, are also parasites of wild and domestic animals. All three species of filaria are transmitted by mosquitoes belonging to the genus *Mansonia*, subgenus *Mansonioides*.

When females of *Mansonia uniformis* Theobald are fed on a cat infected with *Wuchereria patei*, the microfilariae begin to penetrate out from the stomach of the mosquito as soon as the mosquito is engorged and pass into the abdominal haemocoel. They then migrate through fat body cells and the heart forwards into the thorax. This migration is completed within 140 minutes of engorgement. Thus the migration from the stomach to the thorax is rapid, and it is also efficient. When the average intake of microfilariae was 16.5 per mosquito, 98% of the microfilariae ingested were found to migrate successfully from the stomach to the thorax.

Once in the thorax the microfilariae penetrate into the indirect flight muscles and develop only in these muscles. Growth in length does not commence until the third day after the blood meal and by this time the mosquito has developed her eggs. Before growth in length takes place, the insect flight muscle is digested away around the worm but growth in length takes place without the mosquito taking subsequent blood meals. Over 80% of the worms are found to have developed to the infective stage when mosquitoes are dissected nine to ten days after taking in the microfilariae. Thus the majority of the ingested microfilariae develop to the infective stage in the mosquito. Wharton (1957) has demonstrated similar efficiency in *Mansonia longipalpis* Wulp as a vector of *Wuchereria malayi*.

This efficiency of the mosquito in maintaining a large population of developing filariae raises the problem of how the development of the parasite is adapted to the changes in the mosquito host following a blood meal. During the first three days following engorgement fats and proteins presumably from the blood meal are being transferred and deposited in the ovaries under the influence

of an ovarian development hormone initiated from the head (Gillett, 1956). We do not know if the development of the so-called "sausage stage" of filariae in insects is associated directly with the period of ovarian development but we know that the development of the parasite is not dependant on the events that normally follow a blood meal in the mosquito. Female *Mansonia* occasionally do not develop eggs after a blood meal and this usually leads to accumulation of fat in the fat body. Similar fat accumulation is obtained if blood fed females are decapitated within one hour of engorgement, which suggests that fat storage occurs in the absence of the ovarian development hormone. A number of females of *Mansonia* infected with *W. patei* failed to develop eggs but the development of the parasite was normal. Another problem is that the development of the parasite continues in the absence of further blood meals by the mosquito. Possibly the large amounts of amino acids present in insect blood (Florkin, 1949) and known in mosquitoes (Micks & Ellis, 1951, Clark & Ball, 1952) contributes towards the maintenance and toleration of a large number of developing filariae in the mosquito.

Once in the infective stage, filariae in the mosquito maintain their activity for some days. At this time there is a difference in behaviour between the worms related to *W. malayi* and *W. bancrofti*. In the worms related to *W. malayi* there is a much greater concentration of the infective stages in the head and proboscis of the mosquito. This difference in behaviour between the species of the *malayi* group and *W. bancrofti* supports Buckley's view (1960) that the species of the *malayi* group should be put into another genus *Brugia*. It also suggests that the behaviour of *W. bancrofti* during migration and development in the mosquito might be different from that of the species of *Brugia*.

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NOUVELLES OBSERVATIONS SUR LE POLYMORPHISME DES LARVES INFECTIEUSES CHEZ LES MERMITHIDAE

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Les larves néonates infectieuses des Mermithidae présentent une grande diversité d'un genre à l'autre; non seulement elles montrent une grande stabilité de taille alors que les dimensions des adultes sont extrêmement variables, mais encore les traits fondamentaux de leur organisation interne peuvent se ramener à quelques éléments caractéristiques que nous avons déjà définis comme suit (6):

1° une région antérieure, renfermant les organes vitaux (tête, anneau nerveux, œsophage et annexes), dont l'extrémité distale est généralement bien marquée par quelques cellules mères de l'intestin ou des organes génitaux.

2° une région postérieure ou flagellum (f) avec d'énormes vacuoles. Des cristalloïdes se forment à des emplacements assez bien définis. Un groupe de cellules précaudales apparaît à proximité de la queue, celle-ci, de nature cuticulaire, est plus ou moins allongée.

Le flagelle joue le rôle d'appareil locomoteur, il facilite la reptation et aussi la pénétration de la larvule dans le corps de l'hôte, agissant comme propulseur ou comme levier.

Le coefficient flagellaire ($\varphi = f/l \times 100$) donne la grandeur relative du flagellum exprimée en pour-cent de la longueur totale (l) de la larvule.

Nous avons déjà étudié quatre cas extrêmes (6). Une larvule très petite (300 microns) avec un flagellum réduit à peu près aux $^2/_3$ de la région antérieure ($\varphi = 40$) fait regarder *Pseudomermis hagmeieri* Schuurmans-Stekhoven et Mawson (2—7) comme un type primitif pourvu seulement de quatre papilles céphaliques.

Chez *Tunicamermis* (= *Skrjabinomermis*) *melolonthae* Sch.-Stekh., Mawson et Couturier (4—5—7) et chez *Hexamermis* sp. (3) les valeurs des coefficients flagellaires sont voisines (60 à 70) mais les tailles respectives sont très différentes (1000 μ et 3200 μ) et les éthologies dissemblables.

Le genre *Agamermis* est le plus évolué; la valeur de φ est très élevée (85) et le flagelle se détache de la région antérieure au moment de la pénétration de la larvule dans l'hôte, grâce à un dispositif d'autotomisation préformé découvert par Christie (1).

Deux nouveaux exemples précisent l'intérêt de l'étude des larvules infectieuses.

L'espèce décrite sous le nom de *Agamermis couturieri* Sch.-Stekh. et Mawson (7) possède des larvules de taille moyenne: long. 1000—1250 μ, diam. 10—14 μ (au lactophénol 18—20 μ); l/d = 90 à 100. (fig. E)

région antérieure: 650—800 μ, $\varphi = 32$ à 40

tête: long. 30—33 μ, anneau nerveux à 80—100 μ de l'extrémité céphalique, long. 10—13 μ

Oesophage proprement dit: 140—170 μ, à 30 μ de sa partie distale se trouve un amas de noyaux dont la signification n'est pas connue.

La région post-œsophagienne montre surtout des noyaux aplatis et elliptiques (3 × 7 μ); à son extrémité distale, c'est-à-dire de 650 à 800 μ de l'orifice buccal, apparaissent de petits cristalloïdes prismatiques courts, souvent groupés à proximité de noyaux globuleux de 3 μ de diamètre, et dont le nombre varie de 9 à 13. Cet ensemble caractérise le flagellum relativement court (350 à 500 μ) dont l'aspect granuleux apparaît bien sans coloration.

Le diamètre du corps se rétrécit régulièrement depuis le début du flagellum, queue à extrémité arrondie.

Il n'y a pas de nodus, comme l'avait d'ailleurs signalé Schuurmans-Stekhoven et Mawson. — Aussi, en raison de l'absence de cette disposition organique importante, et de la faible valeur de φ, il ne paraît pas légitime de maintenir cette espèce dans le genre *Agamermis*. Celle-ci pourrait appartenir à un genre plus primitif faisant partie du même groupe à pulpe hétérocéphalique.

Chez une autre forme non encore décrite et connue seulement d'après un couple (n° 779 U) (femelle long. 105 mm., diam. 200 μ; mâle 15 mm., diam. 80 μ) les larvules présentent les caractères suivants: long. 750—840 μ, diam. 12—14 μ, l/d = 57—60 (Fig. F).

Région antérieure 350 à 400 μ; flagellum 400 μ, $\varphi = 50$.

Tête long. 23—30 μ, anneau nerveux à 73—90 μ de l'extrémité céphalique, long. 10—13 μ; œsophage: 125—150 μ.

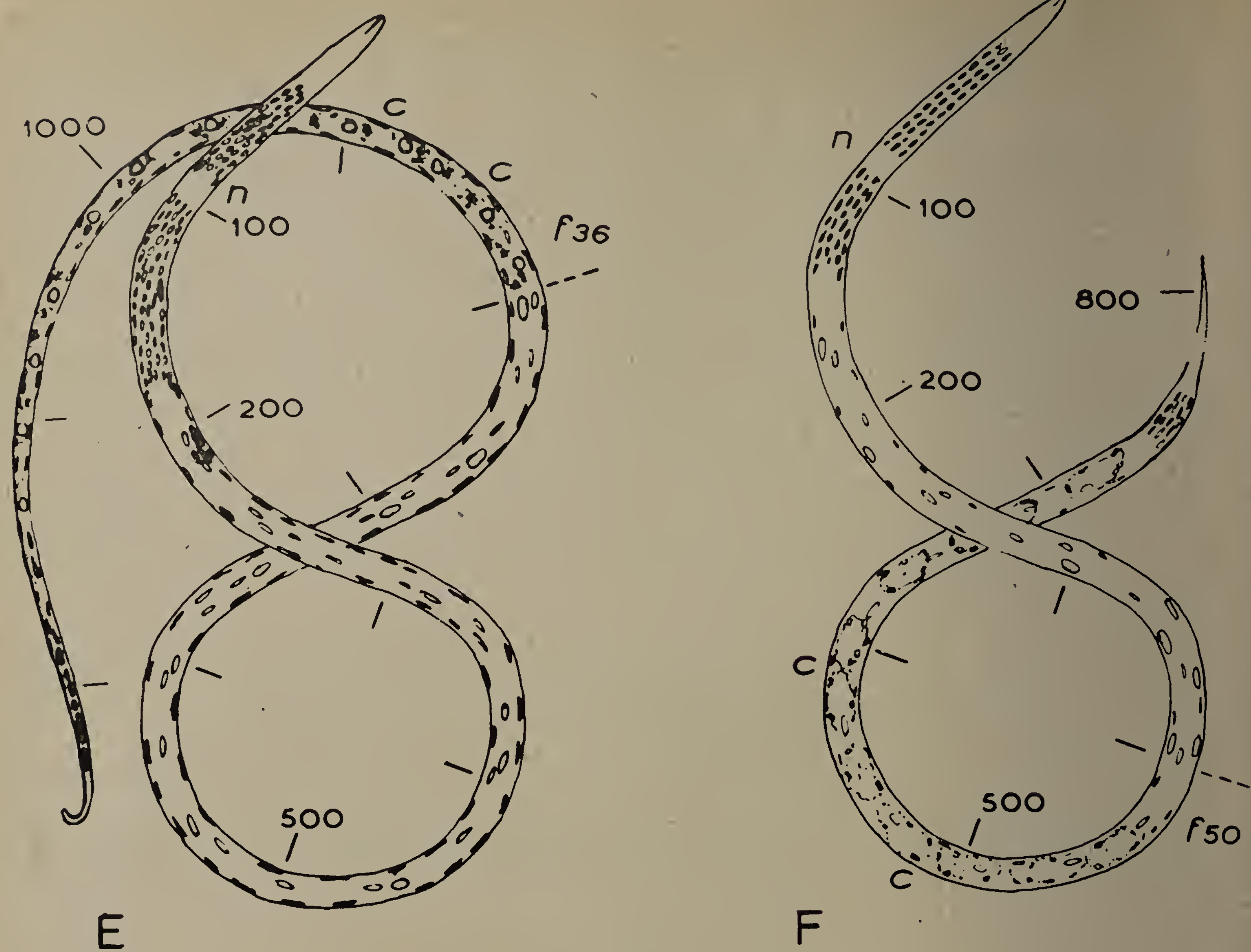

Fig. 1. E. *Agamermis couturieri;* F. 779 U
Les longueurs sont exprimées en microns, la trait tireté est situé entre la région antérieure et le flagelle.
c, cristalloïdes; *f*, flagelle avec, en regard, valeur du coefficient flagellaire; *n*, anneau nerveux.

Flagellum contenant de petits cristalloïdes prismatiques courts. Il se rétrécie brusquement, au-delà du massif de cellules précaudales, en une queue droite effilée de 50—55 μ.

Ces larvules si différentes aboutissent à des adultes d'aspects semblables, parfois de même longueur, les diamètres répondant respectivement à une relation d'allométrie. Au contraire, les larves infectieuses peuvent être différenciées à l'aide de critères précis: coefficient flagellaire, dimensions absolues et rapport de de Man (long/diam). Ces données doivent contribuer à faciliter l'étude systématique de la famille des Mermithidae et aider à comprendre son évolution.

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SOME RECENT OBSERVATIONS ON AFRICAN SIMULIIDAE

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Classification

The Simulium neavei complex

Simulium neavei Roubaud was discovered in May, 1911, and the finding of the early stages by Kenya workers in 1950 opened the way to an adequate study of the complex. The work, however, is often delayed by the difficulty of collecting enough crabs to obtain larvae, pupae and males for identification. Grenier and Mouchet (1959), while describing a new species which occurs in the Cameroon Republic and near Brazzaville, have drawn attention to a microscopic pattern on the larval integument which seems likely to be very useful for classifying other members of the complex. The examination of specimens collected recently in Tanganyika and in the type areas of two species in Nyasaland has resulted in a tentative classification of the complex with special reference to the larval pattern (Lewis, 1961a). The Kenya form hitherto known as *S. nyasalandicum* De Meillon is found to be a new species, and the same is probably true of "*S. woodi* De M." in the same country.

The members of the complex which have been known to bite man readily are *S. neavei*, *S. woodi* in Nyasaland and Tanganyika, and certain forms which are not yet identifiable.

Simuliid larvae

It is seldom necessary to identify first stage mosquito larvae because they are usually found with large ones of the same species. Young simuliid larvae, however, are liable to move downstream, and their identification could give information about their migrations and about the early life of commensal larvae. Crosskey's (1960) work on larval taxonomy refers mainly to large larvae but some of the characters can be used for placing first stage ones in species groups (Lewis and others, 1960).

Distribution

It will take a long time to map the distribution of the *S. neavei* complex, and negative records will be useful. It already appears that the complex is not represented around Inyanga or Salisbury in Southern Rhodesia (Turnbull-Kemp, 1960; Lewis and others, 1960).

Larvae of the *S. neavei* complex have recently been found in a great variety of streams some of which have small reservoirs. There may, therefore, sometimes be a risk of infection with schistosomiasis, and collectors of crabs should consider what precautions they can devise.

Observations in West Africa (Lewis, 1960b) suggest that the distribution of *S. damnosum* Theobald, like that of some Canadian simuliids (Canada..., 1955), is affected by lack of mammalian hosts.

Larvae and pupae

The association between simuliids and crabs

Advantages which simuliids may derive from attachment to crabs have been discussed by Grenier and Mouchet (1958) who referred particularly to current and food supply. Lewis (1960a) suggested that the crabs often save pupae from drying or keep them in sheltered parts of fast rivers. The delicate pupal gills indicate that the complex

may have been derived from a form inhabiting slow streams, and it is likely that the crabs have enabled the simuliids to live in fast ones and thus extend their range. It has been suggested that simuliid larvae on crabs obtain food from their hosts, and the work of Williams and others (1960) is likely to provide information on this subject.

The simuliid-crab relationship is not an isolated phenomenon but one of several aquatic associations (Lewis, 1961b) in which the "epizoite" may be regarded as an occasional passenger, a facultative epizoite, an obligatory epizoite, or a parasite. Certain simuliid larvae which do not belong to the *S. neavei* complex but have been found on crabs (Lewis and others, 1960) were probably occasional passengers and may give some indication of the way in which some regular associations arose. Certain chironomid larvae appear to be facultative epizoites on crabs, and it is interesting to note that both they and larvae of *S. neavei* are usually on the sides of the sides of the host where they are evidently protected by its legs. *S. copleyi* Gibbins may be a facultative epizoite; neither the pupae originally described nor a larva, probably of this species, recently sent from Kungwe in Tanganyika by Mr. D. Eccles and examined by Mr. R. W. Crosskey, were reported to have been found on mayfly larvae, with which this species is often associated. Little or nothing is known of the egg-laying habits of most of the epizootic simuliids and chironomids, but on the other hand the eggs, and no other stage, of a simuliid have been found on a dragonfly larva (Lewis and others, 1960).

Adult flies

Simuliids and trees

Bush clearing was once advocated for controlling *S. damnosum*, but Conran and Conran (1956) report that the inhabitants of the Tonkolili valley in Sierra Leone have learnt to leave riverine bush intact whereas newcomers, who cleared it, have become heavily infected with onchocerciasis. Dry (1921) stated that *S. neavei* was common in thick bush in Kenya, but Lewis (1960a) found that a related species in Tanganyika was rare in forest and common in clearings. The early ideas about bush clearing, however, were based on insufficient knowledge, and Dry's photographs show that he was writing of relatively thin forest. The various published accounts seem to give a coherent picture, and these, together with personal experience, suggest that the following generalisations are permissible. Forest seems to play no part in determining the breeding sites of *S. damnosum* except, perhaps, where thick bush may prevent gravid females from reaching certain streams. Adults of this species can fly a long way in completely open country, they tend to be numerous where there is some vegetation, and are hindered by thick forest and therefore rare in it. Some species of the *S. neavei* tend to breed in or near woodland. Adults of man-biting members are seldom found far from woodland, but, like *S. damnosum*, are scarce in thick forest. It appears that bush clearing can do harm in some areas by increasing the flight range of *S. damnosum*, and good in others by making the environment unsuitable for *S. neavei*. In this connection it is interesting to note that some Canadian simuliids appear to disperse more quickly when forests have been cut (Canada..., 1955).

Biting habits

It is important to know what animals are bitten by simuliids but difficult to obtain gorged females for precipitin tests. Williams and Davies (1957) have collected gorged simuliids with a light trap in Scotland, and Dr. A. Mesghali and I have caught male and female simuliids on sticky traps set for sandflies in Iran. It is hoped that these methods may be of some use in Africa.

A tendency for the proportion of parous individuals, among biting females, to increase about mid-day has been observed in both *S. damnosum* and the *S. neavei* complex

(Lewis, 1960a) but the pattern is very variable and the observed *Onchocerca* infection rate is therefore erratic unless it is based only on parous flies. Such flies are usually easy to recognize but caution is necessary when the fat-body is reduced by starvation or by haplosporidian parasites (Lewis, 1960b).

It is known that *S. damnosum* does not bite man throughout its range. In western Kenya, for example, the species is common but has only been troublesome at Broderick Falls, according to Mr. J. P. McMahon (personal communication). Perhaps only a small proportion of the *S. damnosum* in this region bite man and the species does not make itself felt unless it is abundant. It is difficult to map the distribution of the non-anthropophilic form—the zoophilic race or strain of De Meillon (1957)—because there are many localities, such as the Victoria Falls, where prolonged study might be necessary to find out if there is some extraneous reason for the females not biting.

Control

Need for further study

Apart from the well-known successful eradication schemes there are several areas where eradication seems impracticable and even partial control is beset with various difficulties such as the cost of protecting a sparse human population near a big river. Further studies are required on bionomics (Crosskey, 1959), the action of insecticides, reinfestation of cleared areas (Mattingly, 1958, has discussed this problem in mosquito control), and the minimum control necessary to prevent onchocercal blindness. The last subject is a complex question involving the study of causes of blindness, the various nematodes in simuliids, and the possible existence of an animal reservoir of *Onchocerca volvulus* (Kirk, 1959). In the study of the nematodes, it is difficult to induce captive flies to bite but sometimes possible to find a place and time at which almost all females caught are nulliparous and therefore suitable for feeding on infected animals.

Resistance

Tests for detecting any resistance of larvae to insecticide will sometimes be hampered by the difficulty of obtaining enough larvae and the need to spend time in identifying them. It can take a long time to collect larvae of *S. damnosum* in flooded rivers, or to find many crabs with larvae of the *S. neavei* complex. It is easy, however, to obtain many eggs of *S. damnosum* (Lewis, 1960b) and perhaps of other man-biting species, and the newly hatched larvae might be useful for comparative tests.

Unintentional control

Simuliids are sometimes adversely affected by factors other than planned control measures. At Magombe, in Nyasaland, where the country is too open for tsetse flies, *S. woodi* has become rare, probably as a result of cultivation and bush clearing. In South Africa De Meillon has reported that one or more simuliids have become extinct (Lewis, 1961b). Boroda (1959) believes that industrial pollution of rivers has reduced the numbers of *S. damnosum* around Kilembe in Kenya; and the building of dams in several territories will terminate the breeding of this species in certain localities. Furthermore, there are many rapids where *S. damnosum* is unexpectedly scarce and one or more unknown controlling factors are at work.

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EXPERIMENTELLE UNTERSUCHUNGEN AN STECHMÜCKEN

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Im Rahmen der von Herrn Univ.-Doz. Dr. H. Moritsch, Hygiene-Institut der Universität Wien, durchgeführten Untersuchungen an der Frühsommer-meningo-encephalitis wurden Experimente mit einem Stamm von *Aëdes* (*Stegomyia*) *aegypti* L. aus dem Labor von Geigy, Basel, durchgeführt.

Die hier fortlaufend gezüchteten Mücken wurden von der 6. bis 20. Generation verwendet. Zur Zucht dienen Behälter aus Holzfaserplatten und Glas von etwa $^1/_8$ bis $^1/_6$ m³ Rauminhalt, in die die Puppen eingebracht werden. Als Zwischenfutter wird Honigwasser, 1:7 in Aqua dest. verdünnt, verwendet. 4 bis 6 Tage nach dem Schlüpfen werden große weiße Mäuse, mit Somnifen betäubt, zur Blutmahlzeit vorgelegt. Die Eiablage erfolgt auf feuchtes Filterpapier in Petrischalen, die gleichzeitig der Erzielung eines hohen Luftfeuchtigkeitsgrades dienen. Die Behälter werden nach der ersten Eiablage frisch besetzt. Die Eier werden trocken gelagert und je nach Bedarf in Glas- oder Plastikschüsseln angesetzt. Als Futter für die Larven dient zerriebener Hundekuchen.

Die größten Larven stellen sich bei starker Nahrungskonzentration in sehr seichtem Wasser ein (1—1,5 cm), in stark veralgtem Wasser bleiben die Larven klein. Die Wassertemperatur ist dabei von untergeordneter Bedeutung.

Die Fragestellung war, ob es möglich ist, diese Stechmücken mit Frühsommer-meningo-encephalitis zu infizieren, wie lange das Virus in der Mücke virulent bleibt, ob es sich in der Mücke vermehrt, und ob eine transovarielle Übertragung erfolgt.

Zur Infektion der Mücken dienten weiße Mäuse von 10 g, die mit starker Viruskonzentration „ic“ und „ip“ gespritzt wurden und zum Zeitpunkt der größten Viremie, etwa nach 24 Stunden, mit Somnifen betäubt, zum Saugakt vorgelegt wurden. Für diesen Versuch diente eine Serie von Glaszylindern von etwa 4 l Inhalt, die über einem Zinkblechrahmen stehen, in den eine Lade zum Einbringen von Mäusen, Wasser- und Honigwasserbehälter eingebaut ist. Die Lade wird unter einem Glasdeckel vorgezogen, um zu kontrollieren, daß keine Stechmücke mit entweicht.

In jeden Behälter wurden 60 bis 80 Puppen eingebracht, als Zwischenfutter ebenfalls verdünnter Honig verwendet. 5—10 Tage nach dem Schlüpfen wurden die infizierten

Mäuse 15—30 Minuten lang dargeboten und pro Behälter so 10—30 vollgesogene Weibchen erzielt.

In regelmäßigen Zeitabständen (in verschiedenen Versuchsreihen von 15 Minuten nach der Vorlage bis zu 14 Tagen) wurde jeweils ein Pool mit Äther abgetötet, die vollgesogenen Weibchen zerrieben, in Pufferlösung mit Penicillin und Streptomycin aufgeschwemmt, 10 Minuten bei 3000 Umdrehungen zentrifugiert. Vom Überstand wurde eine Verdünnungsreihe von 10^{-1} bis 10^{-6} aufgestellt und ic.-ip. auf weiße Mäuse verspritzt.

Ebenso wurden Eier von infizierten Weibchen, sowie daraus geschlüpfte Larven, Puppen und Imagines auf Virusgehalt untersucht.

Das Ergebnis brachte einen Virusnachweis in der Mücke von 0—38 Stunden, die quantitative Untersuchung zeigte einen linearen Abfall der Virusmenge. In Eiern, Larven, Puppen und Imagines der F 1 Generation konnte auch mittels Passagen kein Virus nachgewiesen werden.

Es ist deshalb anzunehmen, daß in der Mücke keine Vermehrung des Virus stattfindet, sondern synchron mit dem encymatischen Abbau des aufgenommenen Blutes auch ein irreversibler Abbau des Virus erfolgt. Entsprechende Versuche sollen noch mit Stechmückenarten aus dem Endemiegebiet (Neunkirchen in Niederösterreich) durchgeführt werden.

SYMPOSIUM VIII

THE ECONOMIC STATUS OF PESTS

PEST POPULATIONS THAT MERIT CONTROL

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Summary

All pest control programmes, whether chemical, cultural, or biological, cost money; and the economic cost of a control measure must be equated against the worth of the increased productivity arising from its use.

Recently (Henson, W. R., and Stark, R. W., J. Econ. Ent., 52, 847—850, 1959) pest status has been re-defined: a species may be Tolerable, Critical, or Intolerable in different places in the same year (or in the same place in different years) according to its effect on the host plant. In agriculture the Critical level is too loosely defined as: "the pest population density that destroys more than the excess, but less than the total, host productivity". There is no distinction between Uneconomic populations, which can be shown experimentally to destroy more than the excess host productivity, but not enough to justify a costly control campaign, and Economic populations which seriously affect productivity without killing the host, and which justify artificial control more often than not.

The increasing use of persistent insecticides is causing concern in many parts of the world, and it is sensible to restrict their use to cases where the gain is considerably greater than the potential loss. This thesis implies rejection of routine applications (and in many cases of applications as soon as the first pests are noticed), and the substitution for them of warning systems based on survey information about the pest damage potential in a given area and season.

An understanding of the relationships between pest numbers and host productivity is essential for the development of efficient warning systems, and this contribution discusses some of the published data on pest densities worth controlling, and suggests ways in which such information can be used to reduce the overall cost of pest damage.

THE INFLUENCE OF FERTILIZATION AND IRRIGATION PRACTICES ON FIELD POPULATIONS OF CERTAIN INSECTS OF THE USA

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Summary

It has been amply demonstrated by many entomological investigators that it is possible to obtain responses in certain phytophagous insects by varying the levels of nutrients and/or moisture available to their host plants. Evidence of this has been manifested in laboratory studies by differences in fecundity, length of life cycle and survival among insects grown on plants receiving different nutrient and moisture treatments. Field studies have also indicated that, under certain conditions, fertilizer and irrigation practices may exert a marked influence on populations of several species of phytophagous insects. These influences may be due to a change in attractiveness of the host plants for feeding and oviposition or to other factors yet unexplained. Work by the author has indicated that, under drouth conditions, several species of mirids were more attracted to highly fertilized, irrigated cotton plants than to unfertilized, non-irrigated plants. Later work indicated a three fold increase in the number of *Heliothis zea* (Boddie) larvae found among cotton plants in highly fertilized plots when compared to unfertilized checks. This increase was evidently due to greater attractiveness of the fertilized plants to the ovipositing adults and to the capacity of these plants for supporting larger numbers of larvae.

Certain theoretical aspects of the effects of a community-wide change from a dryland to an irrigated cropping system on population and control of certain insects will be discussed.

COTTON PEST RESEARCH AND THE PEASANT FARMER IN EAST AFRICA

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Summary

The type of research and extension work to be done on the control of any insect pest is very closely related to general farming practice in the area. Whilst insect pests may be studied in detail on local research stations it is necessary to make accurate assessments of their economic importance to the actual growers throughout the district from year to year before general recommendations can be made. In general the results up to date suggest that whilst yields of African-grown cotton could be greatly increased the control of pests, although very important in many areas, is only one factor and would have to be accompanied by much higher standards of cultivation if it were to be profitable. The limiting factor to increasing production in peasant grown crops such as cotton, which is so necessary to improve standards of living, is at present lack of money and the consequent small size of the extension and advisory services available to put into practice information which is already available.

PEST POPULATIONS AND PRODUCTIVITY OF SUGAR BEET

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Summary

Seedling sugar beet is attacked by many pests, and the modern trend towards even lower seed rates with precision drills might lead to more damage before singling. Wireworm (*Agriotes* spp.) was formerly a serious pest, and much is known of population densities likely to cause damage; at present wireworm injury is rare, but 75 per cent of all seed sown is dressed with insecticide as an "insurance". Singling greatly reduces the plant population per acre and pests congregate on the singled plants. Mangold fly (*Pegomyia betae* Curtis) is readily controlled by modern insecticides and action need not be taken until the numbers of eggs or larvae per plant reach a critical level equalling approximately the square of the number of rough leaves.

Later in the season aphids are the only serious pests. In England *Aphis fabae* Scop. does direct damage to the plants, especially in epidemic years like 1959; but *Myzus persicae* (Sulz.) is far more important as a vector of virus yellows. Apart from such cultural measures as early sowing and good plant populations, virus incidence can be reduced and delayed by timely spraying with systemic insecticides. Much data have been obtained in recent years on the relationship between aphid numbers and subsequent virus incidence. Spraying must be done early, when aphid populations are very low, precise timing being decided on local knowledge and experience of such factors as: overwintering sources of virus, aphid numbers, and aphid movement; spray warnings are then issued to growers.

THE INTEGRATION OF BIOLOGICAL AND CHEMICAL CONTROL OF APPLE AND PEAR PESTS IN NOVA SCOTIA, CANADA

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Summary

An integrated pest control programme designed to harmonize biological and chemical control of apple and pear pests has been developed over a period of 17 years. Moderate but significant progress has been made and the programme has been adopted by the majority of growers in the area, resulting in substantial savings in production costs. High quality has been maintained and many of the complications inherent in the extensive use of wide spectrum pesticides have been avoided.

The circumstances under which the change from the general and extensive use of insecticides to the integrated programme occurred involved:

(a) the necessity for economy in production costs;

(b) a co-incident expanding research programme designed to maximize the effectiveness of biological control agents; and

(c) an Advisory Service favourably disposed toward, and enthusiastically conscious of, the possibilities of integrated control programmes.

THE CHEMICAL CONTROL OF THE CARROT FLY (Psila rosae F.) IN GREAT BRITAIN

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Summary

Certain of the chlorinated hydrocarbon insecticides, when mixed with the soil before sowing, give an almost complete control of the carrot fly. The dose necessary varies with the insecticide and with the soil type. The residues of these chemicals disappear slowly from the soil and they still show insecticidal activity after a number of years. Dieldrin is very persistent and half the dose applied has been shown to be present in a peaty soil four years after treatment. When a soil is re-treated with insecticide, allowance should be made for residues present if an accumulation of insecticide is to be avoided. Dieldrin has been shown to be much more effective in controlling carrot fly when incorporated to a depth of 4-inches into the soil than when mixed in more shallowly. Carrots may take up small quantities of insecticide from the soil but, with dieldrin, most of this is found near the surface of the root. High insecticidal efficiency combined with a considerable persistence in soil may lead, with certain of the chlorinated hydrocarbons to the appearance of strains of carrot fly which are resistant to these chemicals. Compounds with a much shorter life and with a different mode of insecticidal action are now being tested to combat such a development.

The example of carrot fly control is used to illustrate the complex effects of modern pest control techniques, and to advocate close liaison between the research worker, advisory officer, and farmer.

DER ENTOMOLOGISCH-PFLANZENSCHUTZLICHE BERATUNGSDIENST IN DER ÖSTERREICHISCHEN LANDWIRTSCHAFT

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Summary

Auftreten und Bekämpfung von Schädlingen unterliegen regionalen Besonderheiten, die auch dem entomologisch-pflanzenschutzlichen Beratungswesen das Gepräge geben. In Österreich sind diesbezüglich folgende Faktoren bestimmend: 1. Beträchtliche Unterschiede in Klima, Landschaftsstruktur und Bewirtschaftung; 2. Mangel an entomologisch und zugleich pflanzenschutzlich ausgebildeten Fachleuten. Aus diesen Voraussetzungen ergibt sich im wesentlichen: a) eine artenreiche und sehr fluktuierende schädliche Insektenfauna, die eine Beschränkung von Forschung und Beratung auf wirtschaftlich vordringliche Probleme erzwingt; b) ein enger Kontakt zwischen Forschung und Praxis, da die Bundesanstalt für Pflanzenschutz — das einzige inländische Institut dieser Art — nicht nur mit wissenschaftlichen und grundlegenden praktischen Fragen, sondern auch mit einer unmittelbaren Beratung der Landwirte befaßt ist.

AGRICULTURAL ENTOMOLOGY IN THE UNITED STATES OF AMERICA

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Summary

The Department of Agriculture has provided the farmers with technical guidance and assistance for many years in meeting the very important agricultural pest problems occurring in the United States. This has been done through its interest in the fields of research, extension, plant pest control, and regulatory work. The responsibility of the Plant Pest Control Division, which is part of this effort, concerns cooperating with the states in the control, eradication, or prevention of spread of a number of plant pests which are new to or not widely distributed throughout their range in the country. In developing an organized control or regulatory program, early detection of the pest is important. Following this, a determination must be made as to the potential importance of the species to American agriculture. In the event a newly introduced pest proves to be one that is capable of extensive damage, steps are taken to set up paralleling state and Federal quarantines which regulate the movement of commodities capable of carrying it long distances through commercial channels. In cases where methods are available indicating the possibility of eradicating the species, steps may be taken to initiate an aggressive program with this objective. This requires a review of procedures and methods which will accomplish the purpose with safety to both operators and consumers. In conducting these operations provisions must be made for keeping the public informed and for a continuing investigation for alternative ways of devising less expensive and more efficient methods.

SOME FOREST INSECT CONTROL PROBLEMS IN THE UNITED STATES AND WAYS TO MEET THEM

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It is well-known that insects cause serious losses to timber, recreational, aesthetic, and watershed values in our forests. Those of us in the United States responsible for reducing such losses have our problems. Moreover, I suspect that our problems are not unique but are instead similar to those confronting pest control personnel of other countries. Some of our problems are perhaps more troublesome than those elsewhere, and perhaps, too, some of them have been getting more than their share of publicity. Of the numerous problems we encounter in insect control, I will discuss only the five I consider most serious. These are: (1) adverse public attitude toward pesticides; (2) when and under what circumstances to undertake insecticidal control; (3) limitations in biological knowledge; (4) prevention of insect-caused losses through forest management; and (5) adequate financing for quick suppressive action.

(1) Adverse Public Attitude Toward Pesticides

Public alarm over expanding use of pesticides and the possibility of undue restrictive action against their use are the most pressing insect control problems in the United States today.

Most entomologists are aware of the propaganda, both for and against pesticides, that has been prominent in communication media of the United States. Some of this propaganda is the distorted expression of extremists. However, there are many honest statements from individuals and groups who seek assurance that the chemicals used will neither be harmful to man nor seriously damaging to other forms of life beneficial to man. I am sure entomologists are in complete accord with this latter attitude. I most certainly am. The public is entitled to and will demand this assurance. Whether we like it or not, the pesticide use problem has become a major national issue. If the average citizen is to get the true story about pesticides—the hazards involved as well as the benefits—we who are intimately connected with the problem have an urgent job to do now. Only the entomologists and allied scientists have the technical information needed to acquaint the public with the importance of pesticides and give assurance that they can safely be used to combat destructive insects. To ignore public opinion is to face the prospect of having our hands bound by restrictive legislation.

Information and education are the principal elements of this urgent job. In intensifying work in the field of public relations, I call particular attention to the following points:

(a) See to it that the public gets the facts about every large insect control project before field action is started. This can be done through news articles, radio, television, magazine articles, talks before property owners, and meetings with civic groups and other organizations and individuals concerned. Particularly important are personal contacts with all property owners in or near the treatment area to tell them why and how the work is to be done and to answer any questions they may have.

(b) Candidly recognize the hazards involved when making these contacts and public releases.

(c) Tell of the research that precedes and constitutes the basis for control; the careful preparation and planning that go into a control activity; the precautions and safeguards taken to protect people, crops, domestic livestock, and wildlife.

(d) Point out the losses that can be expected if an insect outbreak is not suppressed, and stress the attention given to weighing control costs against anticipated benefits.

(e) Explain why control measures other than direct application of insecticides, such as biological control, silvicultural control, or logging, were not employed.

(f) Point out the cooperative aspects of control programs. Explain how individuals and groups, such as local forest pest action committees, participate in planning the action to take.

(g) Organize field trips where representatives of affected groups can see the problem on the ground and obtain firsthand knowledge of how pesticides are used and why it is necessary to use them.

(2) When and Under What Circumstances to Undertake Insecticidal Control

Because of the limitations of cultural and biological methods of attacking an insect outbreak, control by insecticides is at present the principal tool the forest manager has for suppressing many insect outbreaks in the United States. Often this is his only tool, particularly when dealing with outbreaks of defoliating insects. Therefore, when an outbreak is discovered and other measures for coping with it cannot be used, only two alternatives are left—undertake insecticidal control or take no suppressive action.

Decision on what to do about an insect outbreak is, as entomologists know, much more complicated than a fire control decision where the objective is to suppress all forest fires. Seldom is it possible or even desirable to exterminate a native insect, although such action would be considered in the case of a newly introduced and potentially dangerous import. It is not even possible under present-day circumstances in the United

States to try to suppress every insect outbreak. Small outbreaks are constantly developing and many of them subside before causing serious economic damage. To control or not to control must be decided by a careful biological evaluation of each potentially dangerous situation, together with an appraisal of the measures that could be taken to suppress the outbreak. Evaluations involve the damage potential of the specific insect in outbreak status, the trend of the outbreak, the status of parasites and predators, a sizeup of the forest environment, and an estimate of the damage that will occur with and without suppression.

Evaluations also include an estimate of the value of the forest resource threatened, an estimate of control costs compared with anticipated benefits, and an appraisal of the possible damage the insecticide to be used may have upon fish and wildlife. Neither the biological evaluations nor the criteria for determining costbenefit relationships are precise measuring devices. The element of personal judgment still looms large in deciding for or against control. More research is needed to improve upon biological evaluation and to develop and strengthen economic guides for appraising the values threatened by an insect outbreak. We are directing an increasing amount of attention to these aspects of the forest insect problem. To support his decision for or against insecticidal control, the forest manager needs all the assurance he can get from the most knowledgeable sources.

(3) Limitations in Biological Knowledge

For some destructive insects we do not as yet have an effective control. This is equally true whether speaking of direct measures, such as applications of insecticides or biological agents, or of indirect measures, such as modified silvicultural practices. Even with existing controls, gaps in our technical information limit their effectiveness in checking insect damage. The obvious solution to this problem is to expand research and to expand research we must first improve the productiveness and quality of existing research facilities. Prospects for increased attention to forest insect research in the United States are good.

(4) Prevention of Insect-Caused Losses Through Forest Management

The statement is frequently made that if forest managers would apply the silvicultural, logging, and reforestation practices that entomologists know to be effective in minimizing insect development, insect-caused timber losses would be materially reduced. This is undoubtedly true and we in the Forest Service of the United States Department of Agriculture are doing what we can to prevent insect damage to the National Forests by:

(a) directing timber sales to overmature forest stands;
(b) logging insect-infested and high-risk trees, such as decadent and weakened ones;
(c) accelerating cultural practices to improve stand vigor, and
(d) urging managers of other forest properties to do likewise.

To date we have just scratched the surface in this important field of prevention. Roads are essential to proper protection and management of forest properties. But our forest road system is still far short of that needed to insure full forest utilization and to make possible intensive management, particularly in the mountain areas of the West. Furthermore, much of our timber consists of overmature trees, trees that were already growing when the country was first settled. In such stands insect control can be only a holding action to preserve as much as possible of the overmature timber until it can be reached and harvested in an orderly manner. This in itself is a major problem and accounts for a large portion of our present insect suppression effort. Finally, for much

of the forest area of the United States, intensive management is yet in the elementary stages. This is so largely because it is not currently profitable to practice that type of "by the acre" tree farming permitting the forest manager to control stand composition, and undertake the cultural measures prerequisite to a regulated forest. Until the time arrives when intensive forest management is economically possible, the full potential of insect control through prevention cannot be realized.

Accelerated harvesting of existing old-growth timber, coupled with a rapidly expanding forest road-building program, is bringing us closer to the time when a full program of pest control will become a reality in the United States.

(5) Adequate Financing for Quick Suppressive Action

Since forest pests frequently affect properties of several ownerships, problems in financing control often extend beyond the capacities of single owners. Control must then be cooperative. Federal responsibility for leadership and financial aid in combating destructive pests on all forest ownerships is recognized in Federal legislation. The Federal Government bears the full cost of combating pests on Federal forest lands, such as the National Forests and the National Parks. The Federal Government, when requested, may also assist the States and private owners of forest lands with control on their properties on a cost-sharing basis. The usual practice is to limit Federal aid on non-Federal lands to 25% of the control cost. Inasmuch as three-fourths of the total forest area of the United States is in State and private ownership, obtaining adequate financing on non-Federal lands is a formidable problem. Some States have adequate forest pest laws but several fail to recognize the need for some State assistance for control on private lands. Because of this limitation, joint State-private-owner financing often is not forthcoming or cannot be obtained in time to launch an effective control program against a threatening epidemic. In such instances, serious damage may be done to the forest resource through lack of timely financing. The solution lies in the enactment of adequate forest pest control legislation by all the States. Such legislation should provide that the State share responsibility for pest control on private lands. Provision for the unpredictable character of pest trouble should also be made by setting up a contingent reserve so that funds are promptly available whenever control measures become necessary.

In conclusion, I would say to all who know the importance of insecticides and must use them in control work:

(1) Insofar as possible, avoid pest trouble by giving first and full attention to preventive actions;

(2) Take every advantage of logging and biological measures for forestalling and alleviating pest trouble;

(3) Plan and conduct with great care suppression operations that require use of insecticides;

(4) Encourage and support an intensified effort to improve every means of control, and finally

(5) See that the public understands these steps and why they are taken.

If these things are done, I am confident we will go a long way toward transforming current criticism and skepticism into support.

DISCUSSION

T. GREAVES: (a) Are newspapers the best medium for getting the truth of problems. It is usual for newspapers to prefer sensational news. (b) How can you expect 52 competitors of State Governments to agree on any expense to control insects. In Australia we have fewer States and it is hard to reach agreement.

W.V. BENEDICT: Yes, the US press gives its own twist to the news releases we give them. Sometimes they will overstress or otherwise distort the story or play some part of it out of character. None-the-less we use the press regularly and by and large advantageously. Of more direct importance are contacts with property owners and with groups who are in one way or another interested in or affected by an insect outbreak and action to suppress it. We think highly of what is known in the US as local forest pest action committees. These committees are composed of all who have a stake in the problem and what can and should be done. We in the US Forest Service are glad to have the opinions of such groups and give careful study to their proposals in deciding what part the Forest Service will take in the control programm.

H. W. MILES: How far is public pressure a factor in initiating considered control measures?

W.V. BENEDICT: We experience considerable pressures from various groups and individuals in the US who are alarmed about losses inflicted by insects in forests and who want remedial measures taken.

AGRICULTURAL ENTOMOLOGY IN CANADA

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Summary

The reorganization of research in the Canada Department of Agriculture has involved considerable thought and a number of decisions bearing on my assignment in this symposium—planning pest control research on a national basis. Certain requisites for national planning in pest control can be defined and illustrated from our recent experience in organizing the Research Branch of the Canada Department of Agriculture.

A. Planning for Pest Control Research

(1) A basic requisite is the integration of entomological research with other disciplines serving agriculture, to permit a unified attack on pest problems. The new organization has assembled at regional research establishments the entomologists, plant pathologists, chemists, horticulturists, plant geneticists and soil scientists required to make a rational approach to pest problems, and has placed them under one Director. Illustrations of consolidations affecting formerly separated units are the Vineland Laboratory and the Winnipeg Research Station.

(2) Provision of small group of senior scientists to co-ordinate the national program. In the Research Branch, a Program Directorate of six senior officers is assigned this responsibility. Each of these officers has special responsibility for an area of agricultural research and crop protection is one of these. Program control is effected through approval and termination of individual research projects, recommendations on the initiation of new lines of work, deployment of staff and resources, and review of agricultural problems on a regional or subject basis. Illustrations involving pest control are: review of agricultural problems in the Province of Quebec; review of strawberry research in Canada.

(3) Provision of a technical information centre in the complex field of pesticides, to collect, collate and distribute information on the efficiency, persistence, hazard and regulatory aspects of pesticides. To meet this need, the Research Branch has

established a Pesticides Technical Information Office and associated with it a monthly Insecticide Newsletter.

(4) Operational requisites for a national program on pest control are: a taxonomic and identification service for all major groups of economic insects, backed up by systematic research; an advisory service on appropriate methods in experimental design and statistical analysis; an advisory service on methods for the determination of pesticide deposits by both chemical and bioassay procedures. The Research Branch has established such services, not to do the actual analyses required by individual research officers, but to develop and advise on appropriate methods and thus to ensure that valid comparisons can be made throughout the national program.

(5) Provision to ensure the detection of newly-introduced insect pests, and to predict the distribution and population levels of established insect populations. These requisites are met by the location of entomologists in all major areas of agricultural production and by annual surveys to provide bases for predicting the distribution and abundance of specific insects, e.g., grasshoppers.

(6) The need for rational partitioning of effort between the Research Branch, the Provincial agricultural agencies, and the chemical industry in the field of pest control. The Research Branch does not itself undertake actual pest control operations. Our function is to undertake the research and develop the recommendations required for sound pest control programs. Based on our research, we provide advice to Provincial Spray Calendar Committees for instance, and to the Federal Plant Protection (plant quarantine) agency. An example of the latter activity was our participation in the eradication of the oriental fruit moth following an accidental introduction in the fruit growing area of British Columbia. Our policy is to foster increased responsibility and participation by local government on the evaluation of pesticides for local usage. Similarly, our policy is to foster greater participation by the chemical industry in developing data on the efficacy, persistence, toxic hazard, and analytical methods for new pesticides they wish to market for use in Canada.

(7) Finally, a most important requisite for national planning of pest control is, we believe, the protection of certain individuals and Institutes from the pressures for the solution of pressing practical problems, thus to foster a fundamental understanding of our pest problems and accummulate knowledge for a more rational approach to them. The Research Branch has established certain Research Institutes to work mainly on the fundamental aspects of pest problems and pesticides.

B. Planning of Pest Control Research

A basic philosophy in the new Research Branch is to decentralize the planning and conduct of research to the regional laboratories and institutes. Within the broad terms of reference of establishments, and the control exercised by the Program Directorate to effect national co-ordination, the individual establishments are responsible for how they carry out the assigned program. Usually, the proposal and plan for specific research projects stems from the initiative of an individual research officer or small group of officers. However, national work conferences of scientists concerned with specific problems provide the means for the planning of integrated research and often leads to the recognition and initiation of new lines of work. A few examples of research on pest control problems are used to illustrate our current program.

(1) Research on the fate of insecticide deposits on apple to illustrate our need for knowledge of the principles governing the persistence of insecticide deposits. Also to emphasize that data on pesticide "residues" should be collected and used to elucidate the "ecology" of the pesticide in relation to that of the pest, and, incidentally but not primarily, to meet regulatory requirements.

(2) The large-scale experiment on the elimination of cattle warbles with systemic insecticides in an isolated herd in British Columbia to illustrate a project arising from a national work conference.

(3) The national research program on the leafhopper—transmitted aster yellows disease as an illustration of an integrated research effort involving entomologists, plant pathologists and plant breeders.

(4) The research on the mode of action of organophosphate insecticides as an illustration of fundamental physiological and biochemical research leading to rational principles for the development of selective insecticides.

(5) The research on application of the sterile-male technique to control of the codling moth, and/or the discovery of the protective effects of polybutenes against orchard pests, as illustrations of approaches alternative to the use of toxic chemicals for pest control.

FOREST INSECT CONTROL IN CANADA

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Summary

Rational planning of forest insect control requires thorough knowledge of the biology and distribution of pest species. Such basic information on forest insects in Canada is obtained through the research and surveys program of the Forest Biology Division and is used in planning control by biological and chemical methods. Biological control projects are initiated by the Forest Biology Division, which maintains extensive scientific collaboration with other research and service agencies leading to importation of biotic control agents. Success has been obtained in a number of major forest pest problems. In direct control projects, such as the treatment of large forest areas with insecticides under emergency conditions, it is necessary to assess hazard to the forest in terms of pest population trends and tolerance limits of the host species. Priorities for treatment are established on the basis of timber value and degree of hazard. The provincial governments and the forest industry, as the major owners and operators of the forest resource, are responsible for organizational and operational aspects of direct control projects and for financing, but financial assistance may be obtained from the federal government. The Forest Biology Division provides advice on hazard areas, insecticide formulations, and control policy, and studies the results of treatment in terms of pest populations and condition of the forest.

Damage to fish populations in widespread aerial spray projects has emphasized the necessity of considering all resources in the planning of direct control projects. A recently established interdepartmental committee, representing federal government agencies concerned with forestry, forest insects, fisheries, and wildlife, periodically reviews all forest insect outbreaks requiring direct control measures and makes appropriate recommendations to minimize hazards to fish and wildlife resources.

THE TACTICS OF THE PREDICTION AND CONTROL OF THE MOST IMPORTANT PESTS OF AGRICULTURAL CROPS IN THE USSR

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Summary

In the Soviet Union the (composition of the) most important pests have not remained constant, but vary with the agriculture of the country. The following pests were previously of great economic importance *Locusta migratoria* L., *Anisoplia austriaca* Hrb., *Euxoa segetum* Schiff., *Loxostege sticticalis* L., *Phytometra gamma* L., *Rhynchites bacchus* L., *Coenorrhinus pauxillus* Germ. etc. Now they have almost lost their economic importance. New pests have arisen, from those for which quarantine is important, *Diaspidotus perniciosus* Comst., *Hyphantria cunea* Drury, *Gelechia malvella* Hb., *Leptinotarsa decemlineata* Say. In recent years, species of the local fauna have appeared, *Eurygaster integriceps* Put., *Chloridea obsoleta* F., *Hadena anceps* Bkh. and some other pests.

The large scale chemical control which has been carried out has produced sufficient material to indicate the advantages and disadvantages of this method of controlling pests. Its fundamental disadvantage is the destruction of the useful insect fauna which are particularly important in the control of pests in whose dynamics entomophagous forms have considerable importance (*Eurygaster integriceps* Put., *Hadena anceps* Bkh. etc.). However, under conditions of pest outbreak the chemical control method appears to be the only effective means of suppressing the outbreak and of maintaining the pest at its initial stage (e.g. *Locusta migratoria*, *Euxoa segetum* etc.). The importance of the chemical method should not be underestimated as a means of evaluating the economic importance of pests and of losses due to them. Under conditions of concentrated crop stands (monocultures) the role of the chemical method increases still more. The greatest difficulty in the radical decision of problems of plant protection by the chemical method is the length of the times of development of the pests.

Agricultural entomology in our country is faced with the immediate problem of practically destroying some pests completely, e.g. *Eurygaster integriceps*, the numbers of which must be reduced to an economically unimportant level (not more than one larva per sq.m.). A prerequisite for investigating such a problem is a sufficiently complete study of the laws of development and multiplication of the most important pests, and the working out of control methods (*Eurygaster integriceps*, *Locusta migratoria* etc.).

The problem also demands a search for new, ecological, methods of control. These means must be integrated with agrotechnical, chemical, biological and other methods of plant protection. A fundamental change of the conditions of existence of the pest in its environment is the basis of ecological methods of control (reclamation, ploughing, etc.) which should be reinforced by physiological methods against the pest (suppression of fertility, elimination of diapause, etc.).

The resources of entomologists of the USSR are directed towards the working out of a general theory of prognoses and of biological control in the wide meaning of this word.

SYMPOSIUM IX

BODENARTHROPODEN

ETUDE SUR LES COLLEMBOLES DES PRAIRIES CORRESPONDANT A L'ASSOCIATION VEGETALE TRAGOPOGONETO-LOLIETUM DANS LES PYRENEES ESPAGNOLES

D. SELGA SERRA

Parmi les problèmes qu'on s'efforce de résoudre dans l'utilisation de la microfaune du sol comme mesure de sa fertilité, c'est celui de considérer le parallelisme entre la production végétale et la densité de la microfaune; l'étude des données numériques ne permet d'arriver qu'à la conclusion générale et logique que, dans un sol biologiquement actif, la faune a un rôle aussi important à jouer que la végétation.

En vue d'applications agronomiques, il semble plus sûr de tenir compte des aspects qualitatives de la faune, de la constance des espèces et de leurs variations dans un milieu donné. On doit toutefois faire face en zoologie du sol à la très grande difficulté que represente notre connaissance actuelle imparfaite de la faune endogée si variée et nombreuse. Il semble dès lors justifié d'engager des études limitées à un groupe zoologique relativement bien connu et d'essayer de déterminer la valeur indicatrice de ses composants.

Le présent travail c'est consacré à l'étude qualitative et quantitative de prairies appartenant à l'association végétale *Tragopogoneto-Lolietum* et ses variétées. Il sera en suite procédé autant que possible, à la recherche des corrélations entré la faune et les données édaphiques et à une comparaison avec des milieux analogues d'autres pays.

Les prairies qui font l'objet de cette étude sont situées dans les environs de Seo de Urgel (Lerida), entre les terrasses des fleuves Segre et Valira à l'ouest des Pyrénées de Catalogne entre 690 et 800 m. Elles ont été simultanément étudiées des points de vue phytosociologiques et édaphiques depuis plusieurs années en relation avec une exploitation rationelle.

Les prairies de Seo de Urgel

La végétation naturelle de la vallée de Seo de Urgel (terrasses fluviales) serait le chêne, sur les pentes il y avait probablement des pins sylvestres melangées avec le chêne vert, dans les endroits à plein soleil. Des cultures de céréales ont suivi les défrichements et les incendes intervenus il y à un siècle ou deux dans la partie la plus basse de la vallée. Au bord de ces champs, plusieurs plantes du *Mesobromion* se sont installées, auxquelles s'ajoutaient d'autres de la classe des *Molinio-Arrhenatheretea* provenant des bords des rivières et des ruisseaux. Après l'abandon des cultures, les espèces de l'ordre *Arrhenatheretalia* se sont propagées. A la fin du siècle dernier, on à étendu la culture des prairies, avec amendements, irrigation périodique, fauchage et autres soins. Peu à peu, les prairies sont devenues homogènes, tous les échantillons examinés appartiennent à l'association *Tragopogoneto-Lolietum* et ses variations.

La plupart des prairies datent donc de plus que 50 ans, les plus anciennes sont les plus riches en végétation.

Engrais

Fumier de ferme: entre décembre et février; au mois de mars émiettement du fumier au moyen de grosses branches.

Amendements chimiques: en général 300—500 kg de superphosphates à l'ha et parfois aussi de la potasse, sulphate d'ammoniaque 100—150 kg/ha.

Fauchage: trois fois par an, fin mai-premiers jours de juin, fin juillet-premiers jours d'aôut, fin septembre-premiers jours d'octobre, pâturage en automne jusqu'à la mi-décembre.

Irrigation: au printemps, avant la première coupe (facultativement), plusieurs fois en été (souvent chaque semaine).

Pour plus de détails (relevés botaniques, climatologie, carte de la région, photographies etc.) voir P. Montserrat «Contribución al estudio de los prados próximos a Seo de Urgel», Publ. del Instituto de Biologia Aplicada T. XXV, 1957, Barcelona.

Technique

Les échantillons avaient un volume dė 200 à 250 cm³., correspondant à une surface de 60 à 80 cm²., l'extraction de la faune a été faite entre le 1er et 3ème jour apres le prélevement à l'aide d'entennoirs de Berlese Tullgre; après 2 jours les échantillons ont été rechauffés et eclairés avec des ampoules de lo à 15 watts.

Les données numériques de la plupart des échantillons sont des moyennes de plusieurs prélèvements faits au même endroit, le même jour.

Etude des stations

Exp. 2, Tonipal, Mayoral, Seo de Urgel.

Prairie située à l'est de la ville, altitude 690 m., sol à plat très compact et peu perméable restant inondé quelques fois pendant un ou deux jours après les irrigations. La pluie peut provoquer aussi des inondations. Surface riche en fumier peu décomposé. Vie microbienne probablement peu développée à cause des inondations. La rivière Valira dont l'eau est trouble en mai—juin ensuite du dégel dans les montagnes d'Andorra apporte de la boue. La prairie est vieille de plus de 60 ans.

Le sous-sol est à 40 cm. de profondeur. Il y a une bonne diffusion de la matière organique dans tout le profil, qui montre une humification peu avancée, en relation avec l'acidité du sol et l'abondance de fumier.

Cette prairie appartient à l'association végétale du *Tragopogoneto Lolietum multiflori* dans sa variété avec *Chrysanthemum leucathenum* et *Holcus lanatus*, grande quantité de *Festuca rubra* et *Trifolium repens*.

Le rendement de la prairie est de 35.000—75.000 kg. d'herbe fraîche chaque année.

Les Collemboles rencontrés dans l'échantillon de mars 1958, prélevés après épandage du fumier, mais avant celui de l'engrais chimique, ressortent du tableau 1.

Exp. 7, Peret de la torre, Domenjo, Seo de Urgel.

Prairie à plat à côté d'un canal d'irrigation, sol riche en limon et argile, graveleux.

Sous-sol graveleux et perméable, avec nappe phréatique élevée à cause du canal.

Accumulation des sels en surface par suite d'évaporation. Peu humide en surface, normal la rizophère et excessivement humide au fond. Sol jamais inondé. En été on observe des paquets de fumier peu décomposés par manque d'humidité.

Le rendement de la prairie est de 50.000—60.000 kg., d'herbe fraîche/ha.

Amendements: 10.000—30.000 kg. fumier par ha.

Les Collemboles rencontrés dans l'échantillon de mars 1958 après épandage de fumier mais avant celui de l'engrais chimique ressortent du tableau 1.

Exp. 3, Rei, Canut, Montferrer (Seo de Urgel).

Prairie vieille de 60 à 80 ans, à plat situé sur la terrasse du Segre en dessous de l'embouchure du Valira. Altitude 700 m. Le sol en surface est meuble, et reste inondé tout au plus dans des dépressions. L'humidité est normale au printemps et excessive en été pendant l'irrigation, seulement, qui provoque l'acidification.

Tableau 1

	Exp. 2, Tonipal III—1958		Exp. 7, Peret de la Torre III—1958		Exp. 3, Mont-ferrer V—1958	Exp. 6, Guillem de baix V—1958 K$_3$
	sol surface avec fumier	rhizo-sphère	sol surface avec fumier	rhizo-sphère		
Epigées						
Orchesella quinquefasciata.		1				
Sminthurinus elegans	13				23	38
Hemiédaphiques						
Ceratophysela denticulata .		1			10	1
Brachystomella parvula ...	7		1	2		
Folsomia quadrioculata ...					234	
Isotomina bipunctata	1		7	3		
Isotomina thermophila....	140	5		6	3	
Isotoma notabilis	14	2	2		5	
Isotomurus palustris	7			1	46	
Lepidocyrtus cyaneus.....	10					
Lepidocyrtus lanuginosus .						
Sminthurides pumilis	2				4	
Bourletielle sp. juv.......						
Euédaphiques						
Onychiurus fimatus	142	9			42	
Onychiurus hortensis					9	
Tullbergia krausbaueri ...	14	1	7	4	4	1
Tullbergia quadrispina ...					1	
Tullbergia ramicuspis						
Folsomia candida........			2		4	
Folsomides parvulus						
Isotomodes productus			1			
Isotomiella minor					5	
Heteromurus tetrophtalmus						
Heteromurus nitidus			1			
Acariens	482	27		35	419	

Tableau 1 (continuation)

Exp. 5, Casas baratas VI—1958 N_3	Exp. 3, Montferrer XII—1958		Exp. 6, Guillem de bais XII—1958			Molière XII—1958		Exp.1	Parera XII—1958		
	N_3	N_3K_3	N_3	K	N_3K_3	S_1	S_2	rhizo-sphere	N_3	N_3K_3	N_3K_3 (d)
								1		1	
172	3	4	6			30			5	2	
	2	1	1					4	2	8	
3	1					1	1	9	3	8	2
	9	2						1			
			6								
	9	4	2	1		49	4	4	14	10	
147	2					2				2	
	1					1					
	5					3	1	5	1		
			1								
12	2	1	2			52				4	
6											
	17	5	50			1	2	2	22	17	2
		1	25	1	5				3		
			3			1	2				
							1				
	3	1	2			1		1			
			3								
	2										
								1			
148	125	74	95			98	1	94	10	33	7

Sous-sol permeable, graveleux.

Cette prairie appartient à la variété normale de l'association végétale, dans les depressions humides il y a *Cynosurus cristatus*, *Achillea millefolium* et *Rumex crispus*, plantes indicatrices d'endroits fortement pâtures, riches en matière organique, elles sont en general défavorables à la prairie.

Rendement: 65.000—75.000 kg. d'herbe fraîche par an.

Amendements: 30.000—40.000 kg. fumier et 300—400 kg. de superphosphate et 100 kg. de potasse par ha.

Les Collemboles rencontrés dans l'échantillon de mai (sans engrais chimique) et dans ceux de décembre (l'un marqué N_3, amendé au sulphate d'ammonniaque et l'autre marqué N_3K_3, amendé au sulphate d'ammoniaque et au chlorure de potasse) ressortent du tableau 1.

Exp. 5, Casas baratas, Seo de Urgel.

Vieille prairie à plat, sol sableaux et limoneux, jamais inondé à cause de la perméabilité. Humidité normale.

Sous-sol graveleux, très perméable.

La prairie appartient a la variante typique avec *Avena pubescens* et *Canex caryophyllea*, *Dactylis glomerata* et *Poa trivialis*. La luzerne était jaune en mars.

Le rendement de la prairie est de 65.000—75.000 kg. d'herbe en fraîche par ha et par an.

Amendements: pas de fumier en 1958, dans autres années 20.000—30.000 kg/ha, et superphosphate 300—400 kg/ha, potasse 50—100 kg/ha.

Les Collemboles rencontrés dans les échantillons de juin 1958 ressortent du tableau 1.

Exp. 6, Guillem de baix, Porta, Castellciutat (Seo de Urgel).

Prairie vieille de plus de 50 ans, à plat, sol sableux et limoneux sur gravier de la terrasse de Valira, 800 m. altitude. Inondations frequentes à cause d'une couche limoneuse imperméable sur le sous-sol graveleux, par suite acidification.

Dans l'association végétale il y a pénétration des plantes hygrophiles et acidophiles.

Le rendement de la prairie: 65.000—75.000 kg. d'herbe fauchée et de 4000 à 6000 kg. d'herbe pâturée par an et ha.

Amendements: 30.000—40.000 kg. de fumier, 300 kg. superphosphate et 80 kg. de la potasse.

Les Collemboles rencontrés dans les échantillons de mai (marqué K_3, amendé au chlorure potasse) et dans ceux de décembre (marqué N_3, sulphate d'ammoniaque, K_3, chlorure de potasse et N_3K_3, sulphate d'ammoniaque et chlorure de potasse) ressortent du tableau 1.

D. Moliné, près de Segalés, Seo de Urgel.

Jeune prairie, sol en pente, argileux. L'analyse chimique indique une haute teneur en calcium absorbé et suffisamment de phosphore, peu de matière organique.

Sous-sol graveleux.

Amendements: chaque année avec sulphate d'ammoniaque, superphosphate et chlorure de potasse.

Les Collemboles rencontrés dans les échantillons de décembre (surface et en profondeur) ressortent du tableau 1.

Exp., Bondancieta, Parera, Seo de Urgel.

Prairie à plat, surface peu compacte, riche en matière organique. Terrain pas acide. Sous-sol peu perméable.

Amendements: fumier, sulphate d'ammoniaque et chlorure de potasse.

Les Collemboles rencontrés dans les échantillons de décembre (rhizosphère; N_3 sulphate d'ammoniaque; N_3K_3, sulphate d'ammoniaque et chlorure de potasse; couche inferieure N_3K_3) ressortent du tableau 1.

Afin d'obtenir un meilleur aperçu des résultats nous les avons réunis dans le tableau 1.

On est alors frappé par une constance qualitative assez marqué de la faune dans les différentes stations, aussi bien que dans les échantillons se rapportant à différentes expériences. C'est sans doute en rapport avec l'homogénité relative de la végétation qui est partout du type *Trogopogoneto-Lolietum*.

Dans les expériences avec engrais complet (N_3K_3) on constate toutefois un appauvrissement numérique de la faune des Collemboles.

Comparons les échantillons pris le même jour et avant l'épandage de l'engrais chimique, en mars 1958, à Tonipal et Peret de la Torre:

Tonipal: station subissant une grande variation annuelle de p_H (7,4—6,2) et aussi dans son chimisme, il y a cependant toujours beaucoup d'azote, le rapport C/N, se mantient entre 6 à 4,5, le sol est en general très humide.

Peret de la Torre: le p_H est assez constante durant toute l'année (7,4—7,8), tenneur en azote est plus faible que dans la station précédente, le rapport C/N, varie entre 6,7 et 7,3, le sol est généralment sec en surface.

A ces différences édaphiques correspondent des différences dans la faune.

A Tonipal il y a un grand nombre de *Isotomina thermophila* en surface tandis que à Peret de la Torre la même éspèce manque en surface mais est présente en la rhizophère. *Onychiurus fimatus* est très nombreux à Tonipal mais complétement absent à Peret de la Torre, il manque aussi à Guillem de baix, juin, K_3 et décembre, K_3, N_3K_3; en revanche il est nombreux dans cette dernière station, échantillon de décembre, N_3. Cette répartition curieuse pourrait s'expliquer de la façon suivante: on remarque que *O. fimatus* n'est jamais présent si le rapport C/N, dans le sol, s'élève au dessus de 6,5, ce qui signifie pauvreté relative en azote. En effet les populations de l'espèce étaient d'autant plus nombreuses que les échantillons examinés correspondaient à des sols plus riches en azote (Tonipal 142 speciments, Guillem de baix, décembre N_3, 50 spec., Monferrer, mai N_3, 42 spec.).

Dans le tableau, il a été fait une distinction entre trois formes biologiques: espèces épigées vivant sur les herbes basses, espèces hemiédaphiques vivant dans la couche superficielle du sol; et espèces euédaphiques aveugles et dépigmentés, vivant en profondeur. En relation avec leurs habitats préférés, ces trois formes biologiques obéissent chaqu'une à des facteurs écologiques dominants propres.

C'est ainsi que les espèces épigées, telle *Sminthurinus elegans*, dépandent étroitement des influences saisonnières et du fauchage, facteurs qui ont moins d'importance pour les deux autres formes biologiques.

Pour les espèces hemiédaphiques, comme par exemple *Isotomina thermophila*, l'humidité en surface semble être un facteur essentiel.

Les *Onychiurus*, enfin espèces typiquement euédaphiques, sont particulièrement sensibles au chimisme du sol comme nous l'avons exposé ci-dessus pour *Onychiurus fimatus*.

Nous avons aussi compté le nombre total des Acariens, dans chacun des échantillons, il s'agit surtout d'Oribatides. Leur nombre semble suivre en gros les variations numériques des Collemboles hemiédaphiques.

Caractéristiques écologiques des principales espèces

Les caractéristiques suivantes des espèces sont basées sur les observations personnelles ainsi que, par comparaison, sur les indications de la littérature (par exemple, Gisin 1955, Franz 1954).

Ceratophysella denticulata: commun dans ces prairies ainsi que probablement dans les champs, les prairies et les compostes de toute l'Europe.

Brachystomiella parvula: caractéristique de prairies fumées.

Folsomia quadrioculata: espèce banale qui apparait quelque fois en grande abondance, tout en manquant dans autres échantillons sans qu'on puisse expliquer ses variations. Il semble que dans les prairies de la Suisse meridionale l'espèce soit remplacé par *F. multiseta*.

Isotomina thermophila: élement commun dans nos prairies. A notre avis il ne semble pas craindre le fumier, comme le montre l'échantillon de Tonipal, mars 1958, mais fuit la sécheresse du sol superficiel (échantillon Peret de la Torre, mars 1958).

Isotoma notabilis: présent dans presque toutes les stations avec maximum dans celle de Casas baratas, juin 1958.

Lepidocyrtus cyaneus: espèce de surface constante dans nos échantillons; aprés Gisin, frèquente dans les matières organiques en décomposition.

Sminthurinus elegans: constant dans ces prairies, plus rare en Suisse où il est remplacé par *S. aureus*.

Sminthurides pumilis: assez constant et caractéristique des prairies.

Onychiurus fimatus: commun dans ces prairies, en Suisse et Allemagne dans le compost.

Onychiurus hortensis: moins abondant que *O. fimatus* parmi la cinquantaine de spécimens, trouvés dans differents échantillons, aussi bien en printemps qu'en hiver, tous se son révélés être des femelles.

Tullbergia krausbaueri: assez constant et toujours peu abondant d'après Gisin, l'espèce semble favorisée par le fumier.

CONTRIBUTES TO THE KNOWLEDGE OF SOIL FAUNA OF SOUTH-EASTERN ALPS

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I wish to expose the results of a first comparative examination of the soil fauna of three areas of South-Eastern Alps, the Recoaro district (Venetian Prealps), extended from 300 to 1100 m, the Sesto (or Sexten) district, in Pusteria (Pustertal), from 1300 to 2200 m, and the Marmolada massif, Dolomites, from 2000 to 2600 m.

The climate of these three areas is visible from fig. 1. The main features of the climate are furthermore summarized in the following figures:

District	Altitude (in m)	mean ann. temp. (°C)	rainfall (mm)	days with rain	Lang's rainfactor	Gams' index
Recoaro	450	10.3	1855	111	180	13° 18′
Sesto	1518	5.1	920	111	180	59° 2′
Marmolada	2000	3.4	936	89	275	65° 17′

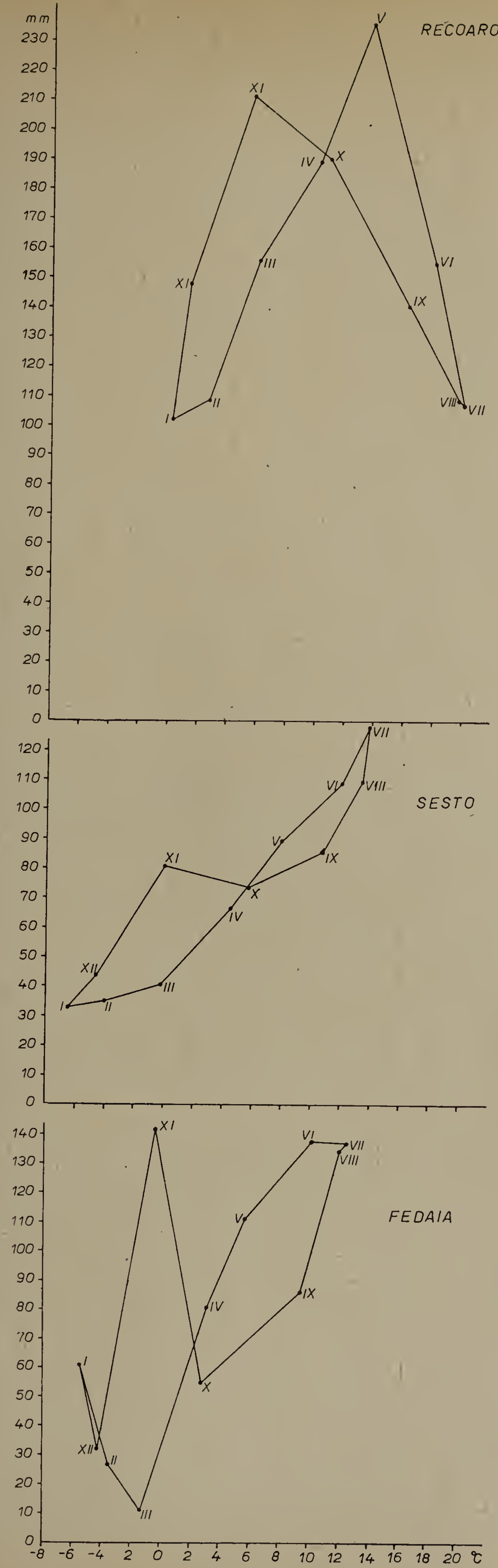

Fig. 1

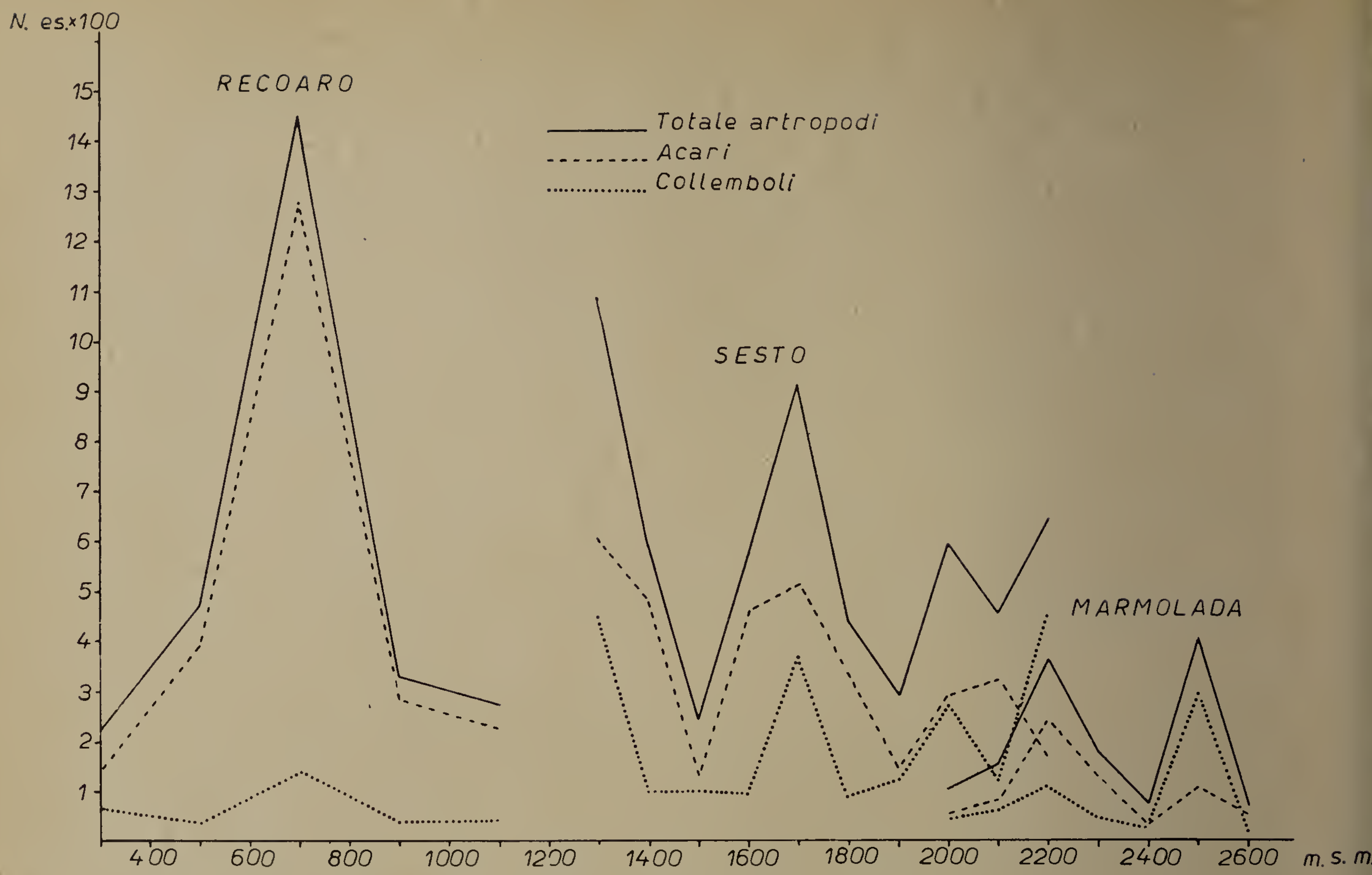

Fig. 2

The soils of Recoaro belong partly to rendsina (soils developed on limestones), partly to chestnut soils ("Braunerde"), developed on igneous rocks; the soils of Sesto belong mostly to rendsina—inclusively of "proto-rendsina"—only secundarily, in the lowest levels, to podsol (coniferous forest). The soils of Marmolada belong at the lowest levels to rendsina and protorendsina, in the highest to raw soils ("Rohboden"), represented by a raw mountain soil, corresponding to Kubiena's "Rawmark" or to "lithosols" of North-American authors.

The data for the Recoaro district are based on the collection of March—April 1959 made by Dr. F. Di Castri; the data for the Sesto region are based on materials collected in July—August 1958; those for the Marmolada are due to collections made during July—August 1952. The lattest have been already published (Marcuzzi, 1959); those on Recoaro are dealt with in a preliminary note by Dr. Di Castri; the data for the Sesto region are not yet published.

The fig. 2 represents the number of individuals per each level (average number of arthropods extracted from 500 cc of soil by means of a Berlese funnel); its examination permits us to draw the following preliminary conclusions.

In the Prealps the summits of the mountains are exposed to strong winds and heavy rainfalls, so that they show "alpin" conditions even at relatively low levels (1100 m in the Recoaro district, 1650 m on the Passo del Broccon). As a consequence of this sharp climatic and environmental change, the soil fauna is much impoverished comparatively with that of forest (chestnut or beech).

The soil fauna of the Sesto region is much richer than that of the Marmolada massif, and even at the same levels (2000—2200 m), perhaps because in the former region elements of the valley can easily reach high levels, due to the particular form of the relief (greater insolation, shelter from cold northern winds, etc.).

The highest density of soil fauna corresponds to 700, 1300, 1700, 2200 (both in Sesto and Marmolada region) and 2500 m. It is difficult at present to say whether these

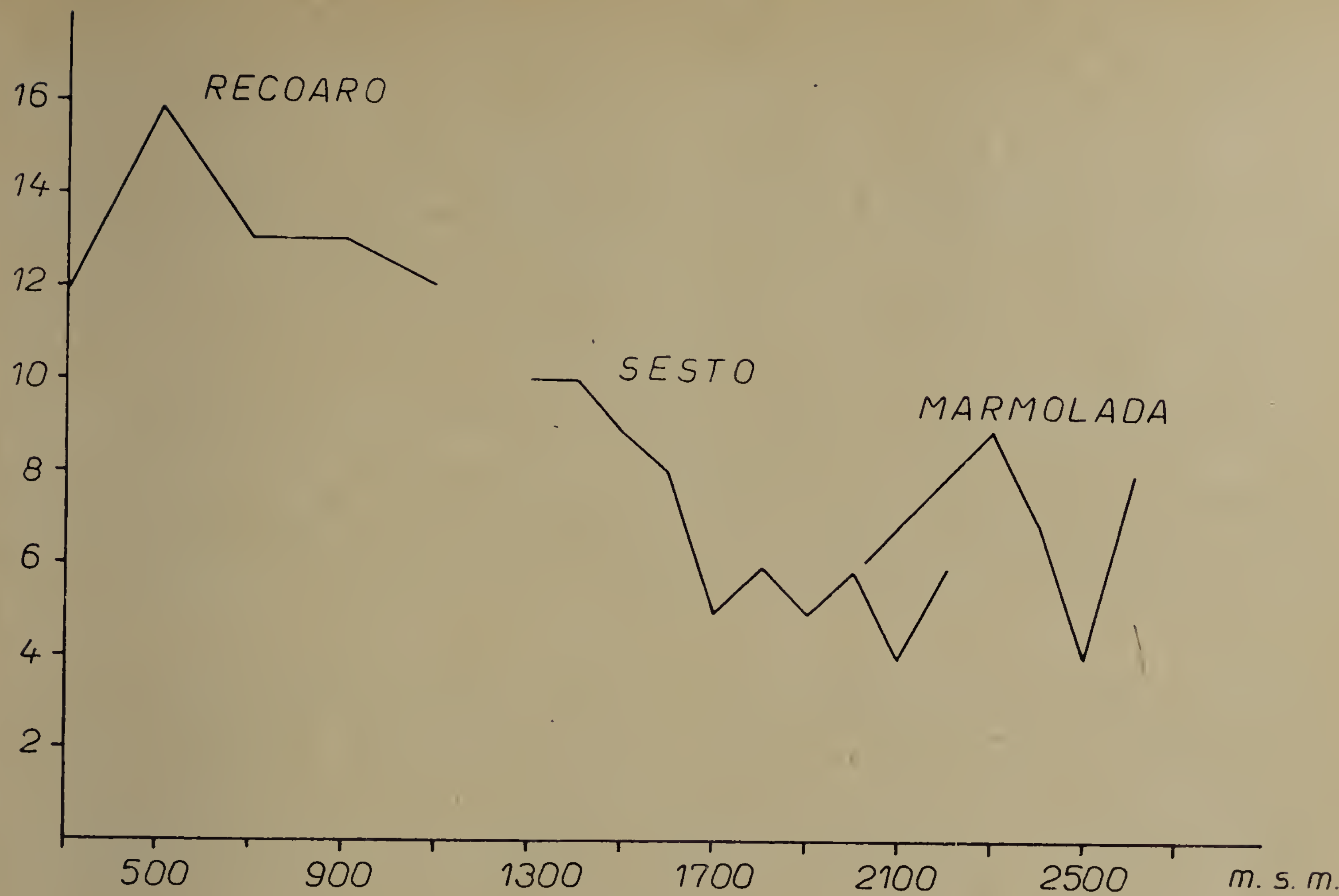

Fig. 3

figures are due to haphazard, or to some definite environmental factor, for which at these altitudes there are some conditions which particularly favour the soil fauna: it is rather suggestive that the values 2500, 2200 and 1700 m have a particular meaning also for the vegetation, representing the maximum development of pionier association (Thlaspetalia), Carex firma-association (Firmetum) and Vaccinio-Piceion.

Very interesting is the comparative examination of the number of mites and collembols at different levels: while in the zone of chestnut (and beech) on the Prealps the mites constantly represent the great majority of the arthropod mesofauna, going from 63 to 88% of the total arthropods, in the subalpine or alpine zone the mites are slightly more represented than collembols, or, at the highest altitudes, as 2200 m in the Sesto district or 2500 m on the Marmolada massif, they are less abundant than those. Of course this will be of paramount importance in the study of the genesis of Italian alpine soils, and more particularly of the humus.

Calcareous soils—at least in the Recoaro region (Prealps)—are richer in number of arthropods than not calcareous ones: this too will have an importance in the study of the evolution of calcimorph soils versus no calcimorph.

When considering the soil fauna from a qualitative stand point, we notice a progressive reduction in the number of the animals groups which constitute the soil fauna with the increase of altitude, as shown by the following figures[1]:

Region	No. of animal groups
Recoaro	22
Sesto	16
Marmolada	11

[1] It is to notice that the zoological groups taken into consideration are not always equivalent from a systematic point of view, since some of them are classes, some orders or even families.

This is still more evident if we examine the number of groups present at each level (see fig. 3). We can conclude from this that the number of zoological groups—which represent the variety of soil arthropod fauna—decreases regularly from 500 to 2100 m, with an exception for the soil fauna of the Marmolada massif, which shows a relatively greater variety when compared with that of Sesto. More exactly, groups of Arthropods present only in the Recoaro district are[2]: Opilionida, Isopoda, Pauropoda, Diplura, Diptera (adults); groups present at Sesto but not on the Marmolada: Thysanoptera, Symphila, Formicidae, Protura, Chilopoda, Psocoptera. No group is present only on the Marmolada, nor at Sesto and not at Recoaro.

Number of specimens and number of animal groups seem to be strictly dependent on the annual temperature of the biotope.

Preimaginal stages of holometabolous Insects are abundant at every level in the Sesto area; in the Recoaro district they increase up to 500 m, then rapidly decrease; the same is true for the Marmolada massif, where the greatest abundance is found at 2400 m.

An examination of the Coleopterous fauna (both quantitative and semi-quantitative samples) in the three areas shows an enormous qualitative differentiation. Indeed, of 70 species present in the territories of Sesto and Marmolada, only 3 (i.e. 3.8%) are common to the two territories, 34 having been collected only at Sesto, 41 only on the Marmolada; of 62 species present at Sesto and Recoaro, only 1 (i.e. 1.6%) is common to the two territories, 37 having been collected only at Sesto, 23 only at Recoaro.

I think we have sufficiently demonstrated the great differences—both qualitative and quantitative—on the composiiton of the arthropod soil fauna of the three areas. The factor mainly responsible for these differences seems to be the temperature. Researches on soil fauna of Recoaro and Sesto are still in progress.

[2] Limiting ourselves to quantitative samples (Berlese's sampler).

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DIE GESETZMÄSSIGKEITEN DER VERBREITUNG VON BODENINSEKTEN UND ANDEREN WIRBELLOSEN IN GEHÖLZEN DER STEPPENZONE OSTEUROPAS ALS KENNZEICHEN DER LEBENSFÄHIGKEIT DER STEPPENWÄLDER

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Die natürlichen Tal- und Schluchtwälder in den Steppen sind als Muster der ständig existierenden sich wiedererzeugenden Gehölze in dieser Zone zu betrachten. Die Bodenfauna solcher Wälder enthält mehrere echte Waldbewohner, deren Auftreten die für das Waldwesen günstigen Bedingungen widerspiegelt.

Das Verhältnis zwischen den Wald- und Steppenarten von Bodentieren in den vom Menschen gepflanzten Forsten, Waldstreifen usw. zeigt, ob die Standortsbedingungen den Forderungen der Wälder entsprechen.

Im Norden der Steppenzone ist die Bodenfauna der besten künstlich gepflanzten Gehölze solcher der natürlichen Wälder ziemlich ähnlich, doch enthält sie auch manche Arten, die mit den Steppengesträuchern verbunden sind. Das zeigt, daß die Bedingungen für das Wachstum solcher Gehölze ziemlich günstig, aber für ihre Selbstwiedererzeugung ungenügend sind.

Im Zentrum der Steppenzone ist der Boden gepflanzter Wälder und Waldstreifen überwiegend von solchen Tierarten besiedelt, die hier, wie auch nördlicher, in den vom Gesträuch bewachsenen Steppenniederungen verbreitet sind, was schwache Lebensfähigkeit solcher Gehölze bezeichnet. Im Süden der Steppenzone bewohnen den Boden gepflanzter Gehölze solche Tierarten, die in den mit Gräsern bedeckten Steppenniederungen verbreitet sind, was als ein Zeugnis ungünstiger Bedingungen für Gehölz dienen kann.

Im SO der Steppenzone (Kastanienböden) sind typische Insekten der Steppen- und sogar Halbwüstenböden in der Streu des Waldstreifens zu finden. Das zeigt, daß hier die Aufforstung ohne spezielle Maßnahmen unmöglich ist. In der Steppenzone treten überall unter der Decke künstlich gepflanzter Gehölze nur jene mesophileren (im Vergleich mit den sie umgebenden Steppen und Feldern) Bodentierarten vor, die die oberen Bodenschichten bewohnen, während die tiefer eindringenden Arten verschwinden. Das entspricht den Angaben von G. N. Wyssotsky, daß die Aufforstung der Steppe die oberen Bodenschichten befeuchtet und die tieferen austrocknet.

Das Verhältnis von Wald- und Steppenarten in der Waldstreu kann als brauchbares Kriterium für die Beurteilung der Lebensfähigkeit der Gehölze in der Steppenzone dienen.

SOIL ENTOMOLOGY IN CANADA—A SUMMARY OF RECENT AND CURRENT WORK

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Soil arthropods in general

Apart from the largely unpublished work of K. M. King and his associates in Saskatchewan in the 1930's there has, until recently, been practically no general soil sampling for arthropods in Canada, although McGuffin made some preliminary investigations in the forest soils east of the Rocky Mountains about a decade ago. Fox in Nova Scotia and Duncan in eastern Quebec have also been taking general soil samples, particularly in connexion with their studies on the effects of insecticides. Some preliminary work is now being done in western Ontario and the author and his associates are currently engaged in various exploratory investigations in respect of the ecology and biology of soil micro-arthropods in south-western Quebec.

Scattered throughout the literature there are numerous passing references to various soil-inhabiting arthropods of many classes and orders from various parts of Canada, including the Arctic, but there would be little point in attempting to review such records. Certain groups are, however, perhaps worth referring to.

Acarina—various works, both by Canadians and non-Canadians, give occasional references to the distribution of soil forms, but the Danish biologist, Marie Hammer's accounts of Arctic Oribatids are about the only ones of any magnitude dealing with soil mites.

Myriapods—Most Canadian records of centipedes and millipedes are scattered, but the recent checklist of the North American Diplopoda by Chamberlin and Hoffman cites all the relevant literature for that group. There are also publications on economically important symphylids in greenhouses.

Protura—only three species have been recorded, all of them from the Northlands.

Collembola—There are several papers dealing with Arctic species, the most recent being those of Marie Hammer and of Mills and Richards. There are also a few observations on south-eastern, Rocky Mountain and British Columbia species. L. E. Wade of Vancouver has been recently concerned with the last.

Coleoptera—Apart from incidental records, the main interest in soil beetles has been in the larvae of Elatridae—Glen, Arnason and, more recently, Eidt are among the authors particularly involved in studying the larval stages.

Diptera—Root maggots (Muscidae; Anthomyiinae) associated with crops have received much attention by numerous workers, the systematics particularly by Brooks. *Tabanidae* also have received and are receiving considerable attention. Current work on bionomics, ecology and behaviour is being undertaken particularly in Alberta and Ontario. Stratiomyid larvae are being studied at Edmonton, Ceratopogonidae at Saskatoon and Empididae in Ottawa.

Miscellaneous Insects—Ants and Braconidae are being studied especially in British Columbia and Ontario respectively. Ant-lion larvae (Myrmeleontidae) and earwigs have also received some attention and there is a large programme of research on field crickets (Gryllidae) at Macdonald College.

Soil pests

It is in this field that research has been most active in Canada. As in most countries the literature is voluminous and it is impossible to review it here. The most important soil insect pests are the root-feeding dipterous larvae, such as cabbage and turnip root maggots (*Erioischia* spp.), onion maggots (*Delia* spp.), seed-corn maggots (*Delia* spp.), Carrot fly (*Psila rosae*), cutworms (larvae of Agrotidae) of such genera as *Agrotis*, *Chorizagrotis* and *Euxoa*, wireworms (elaterid larvae) such as *Agriotes*, *Ctenicera* and *Limonius*, white-grubs (scarabaeid larvae) such as *Phyllophaga* and *Polyphylla*, Tuber flea-beetle (*Epitrix tuberis*), root weevils (*Otiorrhynchus*, *Hylobius* and *Hypomolyx*), and the Clover root-borer (*Hylastinus obscurus*).

There are also many other miscellaneous soil insect pests of lesser importance, such as the European earwig (*Forficula auricularia*) and the Vine phylloxera (*Viteus vitifolii*), all of which are receiving some attention, but they are too numerous to mention individually. Forest pests such as the ambrosia beetles (*Trypodendron* spp.) and sawfly prepupae also hibernate in soil and litter and these have occupied the attention of forest biologists to a very large extent.

Soil insecticides

Among the several methods for the application of soil insecticides which have been developed in Canada may be noted the pioneer experiments in North America by Arnason and his colleagues in the development of seed-dressings. The effects of soil insecticides on the germination and growth of cereal and onion seedlings and upon crop yield and soil composition have also been investigated by several workers. The fate of the insecticides themselves in the soil has also been studied by Proverbs at Macdonald College. Virtually nothing has yet been published (Aug., 1960) on the effects of insecticides on the soil fauna in general, although Fox has done some research in Nova Scotia. Similar work has also been done in eastern Quebec. The effect of spray residues on carabid beetle populations in the peach orchards in southern Ontario is also being investigated by Herne.

Edaphic and vegetation factors and soil arthropods

Probably the most significant published Canadian work concerning the effect of edaphic factors on a single species in the soil refers, not to a true soil animal, but to the Larch sawfly, *Pristiphora erichsoni*, the overwintering larvae of which occur in the soil and are much affected by the water-level in larch swamps. The importance of soil moisture in the distribution of tabanid larvae is also receiving attention in southern Alberta. Differences in soil type have been shown to affect the degree of infestation and distribution of onion maggots in Quebec.

Recent investigations by Fox in Nova Scotia on the influence of soil insects on the floristic composition of grassland, and on the influence of vegetation on the distribution of wireworms, are producing interesting results. Research somewhat similar to the latter is being conducted by Begg in Ontario. At Macdonald College a long-term investigation of the ecology of soil-inhabiting micro-arthropods in woodland and on the influence of these animals on soil formation has begun.

Predators, parasites and diseases of soil arthropods

A number of investigations on the natural enemies of various pests, such as root maggots, wireworms, earwigs and grasshopper eggs have been made, but not much is known about those affecting other soil animals. There is, however, some information on fungus diseases and vertebrate predators. Forest biologists have been engaged for some time on a study of the prey-predator relationships between small mammals and the cocoons and prepupae of injurious sawflies.

Physiology of soil insects

Physiological studies have been mainly confined to the larvae of wireworms (Elateridae) and cutworms (Agrotidae), particularly in respect of their nutritional requirements. The studies of Friend on the nutrition of the onion maggot are also among the most important contributions in this field. In forest entomology, the physiology of ambrosia beetles (*Trypodendron*) hibernating in the soil are being investigated, and Prebble's earlier studies on diapause and related phenomena in the European spruce sawfly, *Gilpinia hercyniae*, should be mentioned.

Techniques

Culture methods—Friend's work, mentioned above, was also important in establishing a technique for rearing dipterous maggots under aseptic conditions, and McClanahan has developed a method of mass-rearing seed-corn maggots (*Delia*

cilicrura) which may be applicable to other dipterous larvae. Several workers have developed means of mass-rearing cutworms (agrotid larvae) and Warren has described how to culture bark- and cambium-feeding root weevils that attack forest trees.

Various methods of culturing mites have been developed by Canadian workers, but not primarily for soil-inhabiting species. However, techniques for rearing micro-arthropods (both mites and collembola) are in use at Macdonald College. The methods are mostly adapted from those previously published; the principal innovation is the use of easily handled, interchangeable, individual culture cells.

Sampling and Extraction—Prebble's work on methods of sampling populations of European spruce sawfly cocoons from soil and litter is well known; a more recent technique, however, involves collecting larvae as they descend to the ground and before they enter the soil to hibernate. Devices for capturing adult insects as they emerge from the soil have also been developed for forest insects and for onion fly.

Details of various sifting and soil-washing machines for removing dipterous larvae and puparia from soil have been published by Lafrance and by Read, and some of these methods may be used for separating various other insects from soil, although they are not fine enough for micro-arthropods. Large-scale flotation methods for wireworm extraction are also in operation, particularly by Burrage in Saskatchewan. In British Columbia a combination of "wet" and "dry" methods is being used by Kinghorn to separate *Trypodendron* beetles from forest soil and litter, about two-thirds of the original bulk of the sample being screened off before a modified flotation method is employed. At Macdonald College the Salt and Hollick apparatus has been modified to render it more efficient and less "messy". Auerbach and Crossley's multiple high-gradient funnel apparatus has also been enlarged and modified.

At Macdonald College also, a rapid-sorting dish for mites and collembola, incorporating millipore filters in lateral wells and a suction trap to draw off the fluid, is now in operation, although it still requires to be thoroughly tested.

Observational Methods—Canadian workers, such as Arnason, Fuller, Sullivan and others, were among the first to use radioactive tracers in the investigation of the movement of soil insects, such as wireworms, root maggots and weevils. Holling has developed a radiographic technique for identifying parasitized, diseased and healthy prepupae within sawfly cocoons.

It may also be appropriate to refer here to the preciptin test which has been used by Fox and McClellan to establish that carabid and staphylinid beetle larvae will feed on wireworms.

In conclusion it might be mentioned that the author has suggested the possible use of endoscopes for the observation of subterranean animals, and the practicability of this is being looked into—so far without marked success.

A fuller account of Canadian work on soil arthropods, citing the appropriate literature, will be published elsewhere: Kevan, D. K. McE., 1961, Soil entomology in Canada—A review of recent and current work. Ann. ent. Soc. Quebec, *6* (1960): 19—45.

FEEDING AND MOULTING IN WIREWORMS

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The feeding and resting and moulting patterns of wireworm development have long been of interest to those concerned with the biology of these insects. A number of workers have reported that there is no pattern of development in the feeding, resting and moulting activities of wireworms. Most of the data presented to demonstrate this belief was massed. Unknown age and sex groups have been combined and interpretations have been made from averages of this data. The result has been a confused picture.

Stone (1941) has shown that the length of larval life of a single species, *Limonius californicus* (Mann) can vary from 5 to 53 months in duration. With such variation it would only be possible to show periodic activity by observing individuals.

In all of these studies where a pattern of development was not observed the length of instar, or development in time, was compared to increase in size or weight. Except where "diapause" occurs this is usually a step by step increase. No effort was made, because of very real difficulties, to measure the amount of food consumed.

The biology and ecology of three species of wireworms have been studied in this laboratory for several years (Kring 1955, 1957, 1959). During this period we became interested in the amount of food consumed as a measure of activity. The technique used in these studies was to estimate the volume of feeding of individual larva for one week periods. Larvae were kept in small plastic dishes. Moisture was held at an adequate level by covering the floor of the individual rearing chamber with sand moistened to 35% of capacity. More or less moisture than this level changed the volume of feeding. High moisture reduced the volume consumed and low moisture increased and then reduced the volume consumed. Chamber, sand and food were all changed weekly. Movement of the larva was measured by estimating the amount of disturbance of the sand. Temperature was maintained at $70 \pm 2°$ F.

Uniform sized disks cut from potato slices were provided as food. By use of an area paper it was possible to estimate the volume of potato slice consumed in the one week period between examinations. Sterilization of the rearing chamber and external asepsis of the larvae permitted long periods of examination without decay of the food.

These studies were first made with larvae of the species *Limonius agonus* (Say) reared from eggs. When the total volume of food consumed by these larvae per instar was plotted against time, a regular increase and then a decrease in volume of food consumed was observed. The period of increase extended for three instars, the volume consumed then radically decreased in the next instar. The volume consumed during the next three instars again increased to a higher level than before. At the end of the third moult the decrease was again observed. These changes were observed beginning with the fourth moult. Prior to this moult the volume of food consumed per instar was so small it was difficult to estimate. In addition, to obtain survival of the larvae it was necessary (Kring 1959) to permit cannibalism during the first instars. While the potato plugs were convenient for volume consumption estimates they were not satisfactory for survival of the larvae. Kosmachevskii (1959) observed that potato was not a satisfactory food for a number of species he has studied. Differential survival and development of larvae reared on several foods has also been observed by Davis (1959). However, during our experiments the larvae once fed animals as food regularly increased in weight and size and appeared normal in activity.

Field collected larvae were reared under the same conditions for periods covering four to seven moults. It was possible to fit the results obtained with these wireworm larvae to the same plan determined from the known age larvae.

Since these observations occurred under constant conditions, it is possible to say that feeding activity is not completely under immediate environmental control but also subject to internal regulation. The regular increase and then decrease in volume of food consumed appears to correspond to annual feeding activity of the larvae.

While all the regulating forces of the environment were not controlled, most of those enumerated by Brown (1959) can be accounted for, except particle radiation. These experiments were repeated several times during the years of study.

Similar studies were made with *Agriotes mancus* Say and *Melanotus communis*. With the *Agriotes* species the results were erratic but were somewhat comparable to these obtained with the *Limonius* species. Diapause as observed by Evans (1944) and Kosmachevskii (1959) occurs during the development of *Agriotes* and may or may not precede moulting. With the *Melanotus* species the pattern was completely different. Under these conditions groups of the larvae would moult in unison or would double moult without feeding. In this species number of moults is definitely not a criterion for gauging development.

By changing either moisture level or temperature or both it was possible to interrupt any of these periods of development to show that they are also still under control of the immediate environment. Later by adjusting the time period to compensate for the interruption it was possible to return to the original pattern of activity for the *Limonius* species.

Evans (1944) working with *Agriotes* spp. illustrated the activity of several individual larvae showing a more or less feeding, resting, and then moulting pattern of development. Kosmachevskii (1960) has recently characterized the stages of development in each instar of *Agriotes litigiosus* var. *tauricus* Heyd.

A number of workers have made excellent studies of either individual species of wireworms or of species that have similar biological requirements. However, from these data others have inferred that this is true or that is true of wireworms.

While there are probably many generalizations that can be made about these insects, it should be remembered that this is a diverse group of animals and that, while they are a morphologically distinct group, it is somewhat more difficult to characterize them biologically. Until more information is gathered it is probably best to consider that the general biological traits that we now assign to the larvae of wireworms are probably the characteristics of either systematic or ecological groups.

With this in mind it is easy to understand how conflicts can arise about the interpretation of the results of work with these animals.

Morphological studies such as those of Becker (1956) on the genus *Agriotes* show both the range and relationship of internal and external morphological characters in this one genus. The range of biological variation could hardly be expected to be less.

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ZUSAMMENHÄNGE ZWISCHEN JAHRESZEIT DER LARVALENTWICKLUNG UND BIOTOPBINDUNG BEI WALDBEWOHNENDEN CARABIDEN

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Die fortschreitende Untersuchung zahlreicher Lebensgemeinschaften erweist, daß es Ubiquisten im strengen Sinne nicht gibt. Keine Art ist innerhalb ihres geographischen Areals gleichmäßig verbreitet. Jede Organismenart besiedelt vielmeht immer nur eine größere oder geringere Zahl der in ihrem Areal vorhandenen Biotope.

Die Ökologie hat heute einen Stand der Methodik erreicht, der es erlaubt, auch die Frage nach den Ursachen dieser ökologisch verschiedenen Verbreitung der Arten anzugreifen. Dazu sind in Verbindung mit Freilanduntersuchungen auch Experimente an Gruppen nahe verwandter Arten mit verschiedener Verbreitung erforderlich.

Unter den bodenbewohnenden Arthropoden haben in neuester Zeit vor allem die Carabiden Beachtung gefunden. Das hat zwei Gründe. Erstens lassen sich bei diesen Tieren mit Hilfe der Barber-Fallen-Methode besonders exakte Angaben über die Verbreitung an verschiedenen Standorten und auch über unterschiedliches Vorkommen auf engstem Raum gewinnen. Zweitens eignen sich gerade Carabiden sehr gut für die in der experimentellen Ökologie so aufschlußreichen Präferenzversuche. Beide Vorteile haben ihre Ursache in der ausgeprägten Aktivität der Carabiden und ihrer Fähigkeit, schnellstens auf Änderungen der Umweltbedingungen zu reagieren. Diesen Vorteilen steht als Nachteil die große Schwierigkeit der Zucht gegenüber.

Ausgehend von Untersuchungen über die Verbreitung von Carabiden in Wäldern, Hecken und Feldern wurden zahlreiche Arten auf ihre Ansprüche gegenüber Temperatur, Feuchtigkeit und Licht getestet (Temperaturorgel, Feuchtigkeitsorgel, Lichtorgel). Stenöke Feldtiere und Waldtiere unterscheiden sich in ihren Ansprüchen gegenüber diesen Faktoren sehr deutlich. Als Beispiel sei das Verhalten von *Abax ovalis* Dft. und *Harpalus pubescens* Müll. gegenüber Temperatur und Feuchtigkeit verglichen. Dabei zeigt *A. ovalis* als Waldtier eine wesentlich niedrigere Vorzugstemperatur (VT) als *H. pubescens*, der typischer Feldbewohner ist. Noch markanter ist das Verhalten in einem 5 stufigen Gefälle der Luftfeuchtigkeit, wo sich *A. ovalis* als hygrophil, *H. pubescens* als ausgesprochen xerophil erweist. Dieses verschiedene Verhalten gegenüber der Feuchtigkeit ist konstant und unabhängig von Temperatur und Jahreszeit. Ähnliches Verhalten gegenüber den beiden Faktoren zeigen von den untersuchten Waldtieren

Abax ater Vill., *A. parallelus* Dft., *Pterostichus niger* Schall., *P. cristatus* Duf., *Molops elatus* F. und *M. piceus* Panz., von den Feldtieren *Agonum dorsale* Pont., *A. mülleri* Hbst., *Calathus fuscipes* Gze., *Broscus cephalotes* L. und *Pterostichus cupreus* L. Weniger typisch verhalten sich die euryöken Feldtiere *Pterostichus vulgaris* L. und *P. madidus* F., deren Verbreitungsschwerpunkt zwar im Feld liegt, die aber auch in größerem Umfang in den Wald eindringen. Diese Arten erweisen sich als feuchtigkeitsliebend und eurytherm. Sie besitzen jedoch eine viel größere Toleranz gegenüber höheren Lichtintensitäten als die Waldtiere. Obwohl die genannten Arten überwiegend Nachttiere sind, ist diese Eigenschaft für das Vorkommen in der offenen Landschaft möglicherweise von Bedeutung, doch sind zur Klärung dieser Frage weitere Untersuchungen erforderlich.

Die verschiedene Verbreitung von Carabiden in den Großlebensräumen Wald einerseits, Feld andererseits läßt sich also auf Grund der gefundenen physiologischen Ansprüche befriedigend deuten. Die entscheidende Rolle fällt offenbar der Feuchtigkeit zu, wichtig ist daneben die Temperatur, und auch das Licht scheint in manchen Fällen für die Biotopbindung von Bedeutung zu sein.

Viele waldbewohnende Carabiden kommen nur in bestimmten Waldtypen vor. Besonders auffällig ist, daß viele Formen an Wälder vom Typ der Fagetalia (Edellaubwälder mit reicher Bodenvegetation auf neutralen Böden) gebunden sind. Seltener sind Arten, die nur oder überwiegend in bodensauren Wäldern vorkommen. Eine dritte Gruppe von euryöken Arten besiedelt Wälder aller Typen. Unsere Messungen ergaben nun, daß die Waldgesellschaften der Fagetalia kühleres und feuchteres Mikroklima besitzen als die bodensauren Waldtypen (Quercetalia roboris-petraeae). Es mißlingt jedoch der Versuch, die charakteristischen Carabiden-Arten der Fagetalia durchgehend gegenüber den euryöken Waldbewohnern in Präferendumversuchen zu differenzieren, wie dies bei den Wald- und Feldbewohnern möglich ist (Abb. 1). Von den Arten der Edellaubwälder sind *Abax parallelus* und *A. ovalis* nur etwas hygrophiler als die euryöken Arten, zum Beispiel *A. ater*. Arten wie *Pterostichus nigrita* F. und *Agonum assimile* Payk. sind sogar stärker xerophil als die euryöken Waldtiere. Von den Bewohnern der Fagetalia zeichnen sich nur *Agonum assimile*, *Molops elatus* und in geringerem Maße *M. piceus* durch niedrige VT aus. Die anderen charakteristischen Arten der Fagetalia (*Abax parallelus*, *A. ovalis* und *Pterostichus nigrita*) haben ähnliche VT wie die euryöken Formen. Eine Bevorzugung von niedriger Temperatur und hoher Feuchtigkeit läßt sich also für die Arten der Edellaubwälder keineswegs durchgehend feststellen. Auch das Verhalten gegenüber dem Lichtfaktor kann die Stenökie dieser Arten nicht begründen. Für ihr begrenztes Vorkommen mußte daher eine weitere Erklärung gesucht werden. Auf Larsson (1939) geht die Feststellung zurück, daß es bei den Carabiden Arten gibt, die sich im Herbst fortpflanzen (Herbsttiere) und solche, die ihre Vermehrungsperiode im Frühjahr haben (Frühlingstiere). Frühlingstiere machen die Larvalentwicklung im Sommer durch, Herbsttiere im Spätherbst, Winter und Frühling. Bei den Frühlingstieren sind zwei Gruppen zu unterscheiden: Frühlingstiere mit Herbstbestand, bei denen die neue Generation im Herbst aktiv wird, sich dann aber noch nicht fortpflanzt (dies geschieht erst nach Überwinterung im Frühjahr) und Frühlingstiere ohne Herbstbestand, bei denen die neue Generation im Herbst zwar schlüpft, aber in der Mehrzahl die Puppenwiege nicht verläßt. Es fällt nun auf, daß die für die Fagetalia charakteristischen Carabiden überwiegend Frühlingstiere sind, nämlich nach unseren auf Fängen mit Barber-Fallen basierenden Feststellungen *Abax ovalis*, *A. parallelus*, *Molops elatus*, *M. piceus*, *Agonum assimile*, *Pterostichus nigrita* und weitere Arten (Abb. 2). Für eine Reihe von Arten konnte die Fortpflanzung im Frühjahr nicht nur wie bisher aus dem Auftreten der Imagines und Beobachtungen der Kopula erschlossen, sondern auch durch die gelungene Aufzucht nachgewiesen werden (*Pterostichus oblongopunctatus* F., *Pterostichus nigrita*, *Agonum assimile*). Dabei wurde die Ei-

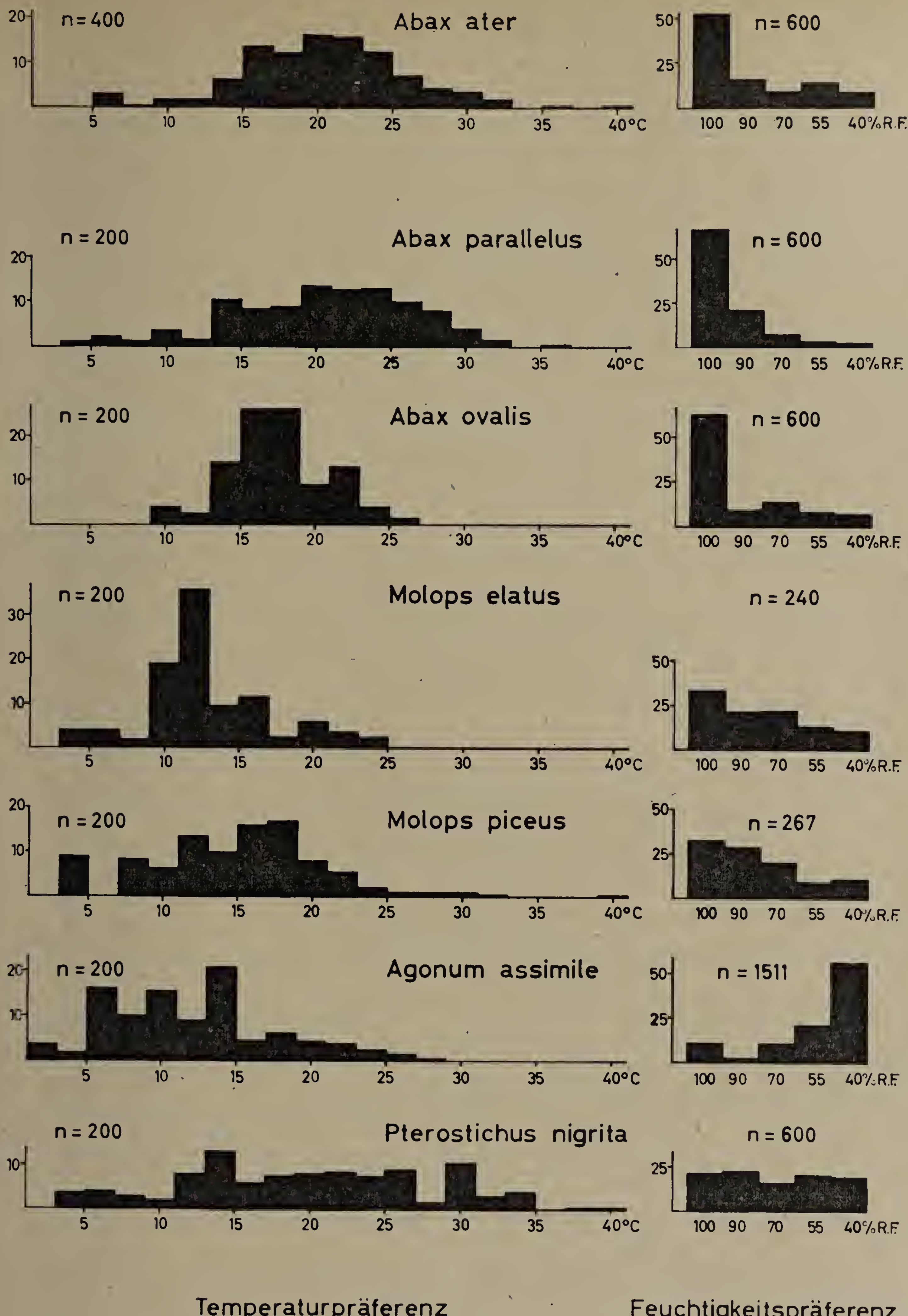

Abb. 1. Temperatur- und Feuchtigkeitspräferenz bei einem euryöken Waldcarabiden (Abax ater) und sechs stenöken Arten der Fagetalia. Ordinate: Aufenthaltswahl in % aller Registrierungen.

ablage von frisch gefangenen Tieren erzielt, so daß eine Verschiebung des Legetermins durch die Gefangenschaft nicht in Frage kommt.

Es sind dies also die Arten, die Larven im Sommer (etwa von Mai bis Juli) haben. Untersuchungen an *Pterostichus vulgaris* zeigen nun, daß das Feuchtigkeitsbedürfnis

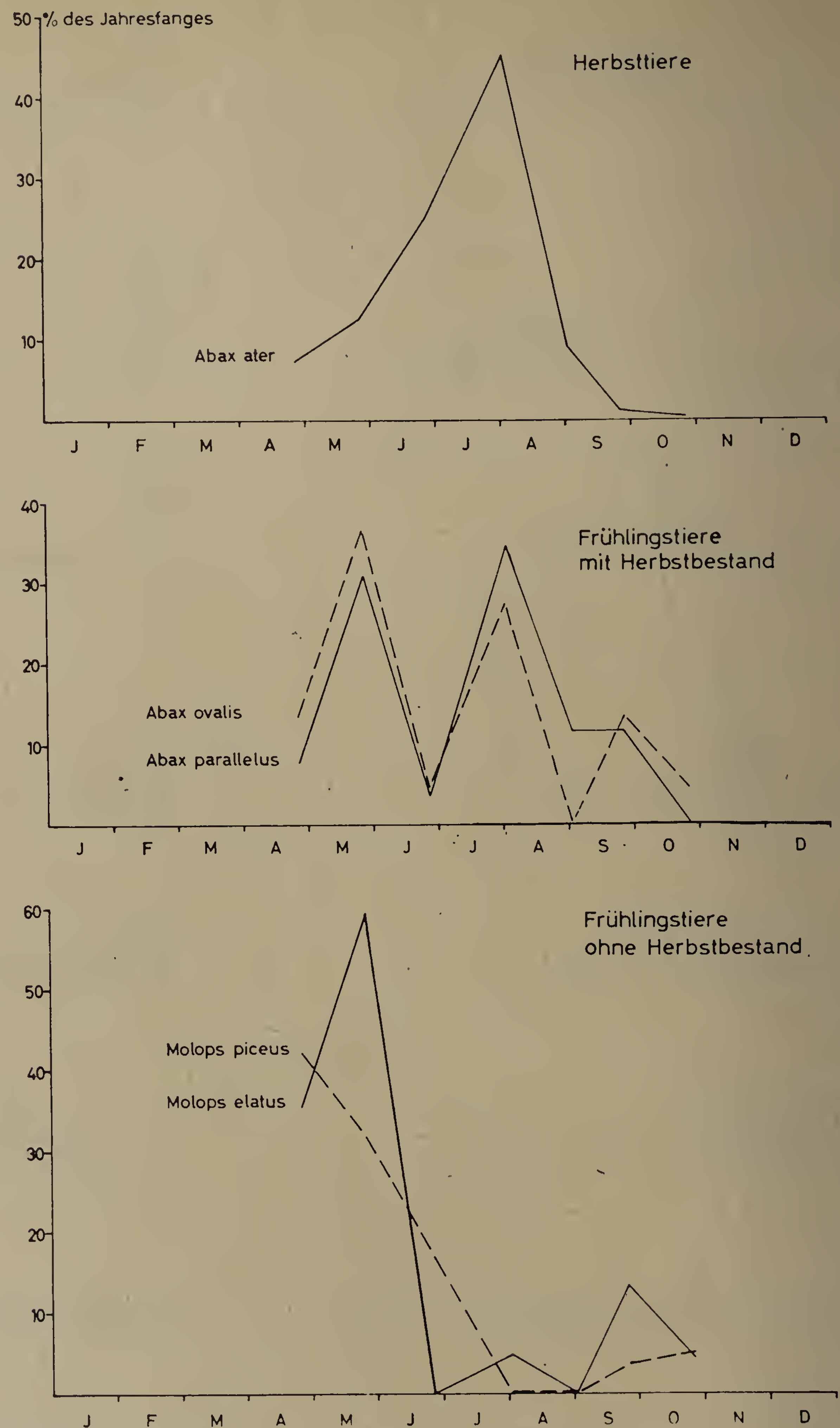

Abb. 2. Beispiele des jahreszeitlichen Auftretens waldbewohnender Carabiden (nach Fangergebnissen in Barber-Fallen).

der Carabidenlarven wesentlich größer ist als das der Imagines. Es liegt daher der Schluß nahe, daß die Bindung bestimmter Arten an die Fagetalia darauf beruht, daß ihr empfindlichstes Stadium in den Sommer (also die Zeit stärkster Einwirkung von Hitze und Trockenheit) fällt. Sie können daher nur an Standorten mit besonders kühlem und feuchtem Mikroklima leben, wie es in den Fagetalia gegeben ist. Dagegen können

Arten mit Larvalentwicklung in der kühleren und feuchteren Jahreszeit euryök sein, wie z. B. *Abax ater*.

In den bodensauren Wäldern kommen Frühlingstiere mit Ausnahme einer Art nicht vor. Diese Ausnahme ist *Pterostichus oblongopunctatus*. Obwohl typisches Frühlingstier, ist diese Art ein eurytoper Waldbewohner, dessen Verbreitungsschwerpunkt gerade in den bodensauren Wäldern liegt. Abweichend von allen anderen Frühlingstieren unter den waldbewohnenden Carabiden erweist sich diese Art im Experiment als eurytherm, xero- und photophil. Diese Eigenschaften der Imago erklären die Bevorzugung der lichten, warm-trockenen bodensauren Wälder. Wie die Larven dieser Art hier die sommerliche Jahreszeit überdauern, muß noch untersucht werden.

Zu erwähnen ist ein Befund, zu dem Larsson auf ganz anderem Wege gelangt ist. Auf Grund einer statistischen Auswertung von Museumsmaterial stellte Larsson fest, daß der Anteil der Frühlingstiere in feuchten Wäldern besonders hoch und in Nadelwaldanpflanzungen (also trockenen Wäldern) verschwindend gering ist, ohne daß er diese Erscheinung erklären konnte. Der Befund stützt jedoch die hier vorgetragene, auf Fallenfänge, Zucht und experimentelle Untersuchungen gegründete Auffassung.

Die Verbreitung der Carabiden kann also keineswegs immer aus den experimentell ermittelten Präferenzbereichen der Imagines erklärt werden. Die Untersuchung der zeitlichen Einpassung der Fortpflanzung und des Auftretens der empfindlichsten Stadien in den Rhythmus der Jahreszeiten ist besonders für die Erklärung feinerer Unterschiede in der Bevorzugung der Lebensräume unerläßlich.

NAHRUNGSWAHL BEI BODENARTHROPODEN IN PRODUKTIONSBIOLOGISCHER SICHT

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Bei produktionsbiologischen Untersuchungen an Bodenarthropoden verhindert oft die noch immer ungenügende Kenntnis von der Nahrungswahl dieser Tiergruppe eine umfassende Auswertung der Ergebnisse. Es wurde daher die Frage aufgeworfen, welche allgemeinen Schlußfolgerungen aus den bisher hierüber angestellten Ermittlungen gezogen werden dürfen. Diese außerordentlich komplexe Frage soll hier auf die Untersuchung der Nahrungswahl laubstreuzersetzender Bodenarthropoden beschränkt werden, da diese experimentell am besten nachprüfbar ist und deshalb auch am häufigsten untersucht wurde.

Vom Zersetzungsprozeß des Laubblattes ausgehend, ist zwischen Erstzersetzern, die das frisch gefallene Blatt angehen können, und Zweit- oder allgemeiner Folgezersetzern zu unterscheiden. Vom Tier selbst ausgehend, bietet diese Unterscheidung jedoch Schwierigkeiten, da weder Erstzersetzer obligatorisch unzersetzte organische Substanz noch Folgezersetzer notwendig zersetzte Substanz fressen müssen. Es ist hier besser, der gültigen Definition des Humus als tote organische Substanz entsprechend zwischen Makro- und Mikrohumiphagen zu unterscheiden, wobei die räuberischen Bodentiere schon begrifflich ausgeschieden sind. Zu den Makrohumiphagen zählen Vertreter der Makro- und Megafauna, die infolge der Größe ihrer Mundwerkzeuge auf größere, zusammenhängende Stücke organischer Substanz (Makrohumus) angewiesen sind, wie sie z. B. ein Stück Fallaub bietet. Sie sind somit vorwiegend Erstzersetzer, bevorzugen aber eindeutig durch Mikroorganismen bereits

veränderte Nahrung, so daß sie unter geeigneten Umständen durchaus zu Folgezersetzern im energetischen Sinn werden können. Soweit die Makrohumiphagen endogäisch leben, graben sie sich aktiv durch den Boden, wobei auch Arthropoden, besonders Diplopoden, nach Art der Lumbriciden Boden in sich aufnehmen und als Nahrungsquelle ausnützen können.

Zu den Mikrohumiphagen zählen dagegen Vertreter der Meso- und Mikrofauna, unter den Bodenarthropoden also vor allem die Apterygoten und Milben. Sie sind infolge der Kleinheit ihrer Mundwerkzeuge in der Lage, Mikrohumus-Bestandteile auszuwählen. Somit sind hier Speisezettel und Spezialisierungsmöglichkeiten viel umfangreicher. Hinsichtlich der Blattzersetzung sind sie meist Folgezersetzer, ernähren sich also von Kotballen der Erstzersetzer oder von mikrobiell stark veränderter organischer Substanz. Sie können aber auch als Erstzersetzer auftreten, wie Fütterungsversuche mit frischem Fallaub zeigten. Wohl alle Mikrohumiphagen nehmen neben toten organischen Bestandteilen auch Mikroorganismen auf; bei einer Anzahl von Arten scheint sogar eine ausschließliche Spezialisation hierauf eingetreten zu sein. — Es bedarf keiner Erwähnung, daß es zwischen den Gruppen der Makro- und Mikrohumiphagen Übergänge gibt, insbesondere bei Jugendstadien.

Am besten bekannt ist die Nahrungswahl streuzersetzender Makrohumiphagen. An verschiedenen Diplopoden- und Isopodenarten wurden ausgedehnte Fütterungsversuche mit frischem Fallaub einiger wichtiger mitteldeutscher Laubbäume angestellt. Es war keine Spezialisierung einer Tierart auf eine bestimmte Blattart festzustellen, sondern alle Tierarten verhielten sich grundsätzlich euryphag. Dabei zeigten die geprüften Arten eine erstaunlich einheitliche Reihenfolge der Bevorzugung, so daß eine allgemeine Präferenzreihe für frisches Fallaub aufgestellt werden kann. Die Anordnung der geprüften Blattarten lautet: Winterlinde, Esche, Schwarzerle, Feldulme, Bergahorn, Spitzahorn, Hainbuche, Stieleiche, Rotbuche. Dieser Anordnung entspricht auch die Reihenfolge des Verschwindens bzw. der Zersetzung der Blattarten unter natürlichen Bedingungen im Waldboden.

Bei entsprechenden Versuchen mit überwintertem Fallaub fallen die durchschnittlich größeren Fraßmengen und die gleichmäßigere Verteilung des Fraßspektrums auf. Grundsätzliche Unterschiede zu den vorher besprochenen Ergebnissen zeigen sich jedoch nicht. In der Präferenzreihe selbst ergibt sich eine Änderung dadurch, daß die überwinterten Hainbuchenblätter relativ mehr bevorzugt werden als die frischen, so daß die Hainbuche von der 7. auf die 4. Stelle der Präferenzreihe rückt. Bemerkenswert ist, daß diese geänderte Bevorzugung wiederum sehr gleichmäßig bei den geprüften Tierarten auftritt.

Die geschilderten Ergebnisse legen nahe, die streuzehrenden Makrohumiphagen als euryphag mit gleichgerichteter Nahrungswahl zu bezeichnen. Die Anerkennung einer solchen Schlußfolgerung hängt zunächst davon ab, ob sich die Ergebnisse derartiger Fütterungsversuche, die bekanntlich mit einer großen Schwankungsbreite behaftet sind, statistisch sichern lassen.

Abbildung 1 zeigt die Sicherungsgrenzen der Ergebnisse eines solchen Präferenzversuches an dem Diplopoden *Julus scandinavius* mit frischem Fallaub von 20 Blattarten. Die variationsstatistische Prüfung dieses Gesamtversuches ergab eine einwandfreie Signifikanz der Fraßunterschiede gegenüber den versuchstechnischen Fehlerquellen. Aus der Darstellung sind die Sicherungsgrenzen der Bevorzugung der Blattarten gegeneinander nach den Grenzwahrscheinlichkeiten (P) zu ersehen. Erkennt man Unterschiede mit der Grenzwahrscheinlichkeit $P < 5\%$ (Halbkreis in Abbildung 1) als gesichert an, wie dies in landwirtschaftlichen Feldversuchen mit ähnlicher Schwankungstendenz üblich ist, so ergibt sich eine gruppenweise abgestufte Sicherung der Ergebnisse. Als Beispiel sind die Sicherungswerte für die oben erwähnten Blattarten

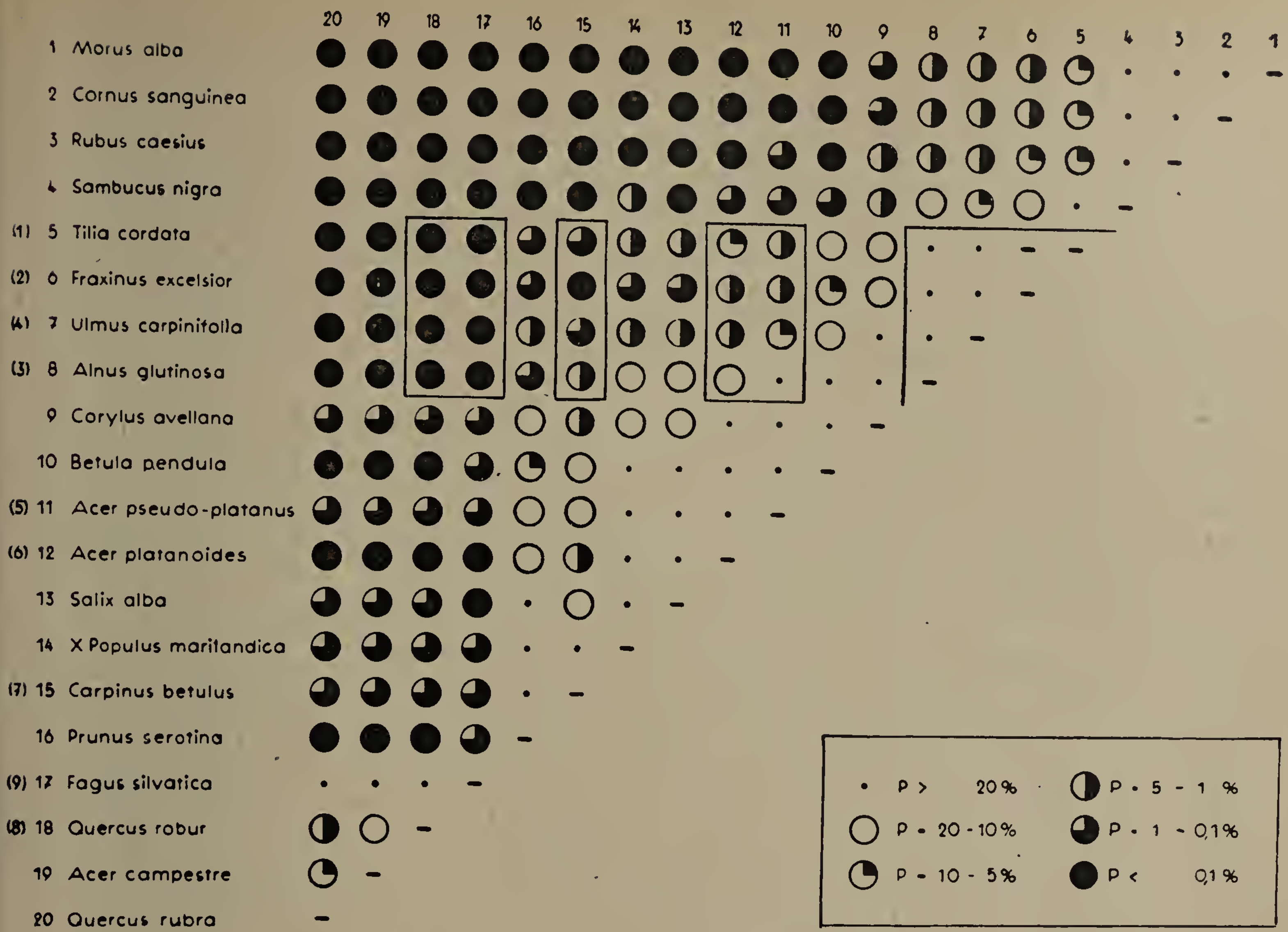

Abb. 1. Präferenzversuch an *Julus scandinavius* (*Diplopoda*) mit frischem Fallaub: Darstellung der Sicherungsgrenzen des Nahrungswahlvermögens nach den Grenzwahrscheinlichkeiten (P). Die waagerechten Reihen geben Bevorzugung, die senkrechten Ablehnung an.

besonders markiert. Die eingeklammerten Zahlen links geben die Stellung dieser Arten in der zuerst gegebenen durchschnittlichen Präferenzreihe an. Im hier vorliegenden speziellen Fall zeigt es sich, daß die Reihenfolge im Vergleich zur Durchschnittsreihe zweimal um eine Stelle verschoben ist. In beiden Fällen liegen aber die P-Werte weit über 20%, so daß die Bevorzugungsgrade bei beiden Blattpaaren als statistisch gleichwertig angesehen werden dürfen. Im einzelnen ist zu erkennen, daß die bevorzugten Blattarten Winterlinde, Esche, Feldulme und Schwarzerle eine Gruppe bilden und unter sich keine gesicherten Unterschiede aufweisen. Diese Arten werden durchweg sehr gut gesichert bevorzugt vor Rotbuche und Stieleiche, sehr gut bis genügend gesichert bevorzugt vor Hainbuche (frisches Fallaub!) und nur knapp oder im Fall der Schwarzerle ungenügend gesichert bevorzugt vor Bergahorn und Spitzahorn. Die fehlerkritische Analyse der Ergebnisse ergänzt also die quantitative Auswertung der Fütterungsversuche dahingehend, daß eine gesicherte gruppenweise Abstufung der Bevorzugungsgrade vorliegt.

Es ist weiter zu prüfen, ob diese im Labor erhaltenen Ergebnisse auch unter natürlichen Verhältnissen ihre Gültigkeit haben. Die Methode der Direktbeobachtung stößt besonders wegen der nächtlichen und verborgenen Lebensweise der in Betracht kommenden Arten auf große Schwierigkeiten. Die sichersten Ergebnisse sind durch Untersuchung des Darminhaltes frisch gefangener Tiere im Vergleich mit dem Darminhalt von Versuchstieren mit bekannter Nahrung zu erhalten. Man hat dabei nach ähnlichen Methoden zu arbeiten, wie sie in der Pharmakognosie für die Prüfung von Drogen angewendet werden. Derartige Nachprüfungen an verschiedenen Standorten unter mitteldeutschen Verhältnissen bestätigten die Gültigkeit der experimentell

gefundenen Blattpräferenz unter natürlichen Bedingungen. Sie zeigten aber auch, daß je nach den ökologischen Ansprüchen der geprüften Art bzw. den Gegebenheiten des Standortes das effektive Nahrungsspektrum stark wechselt. Während die Bestimmung der gefressenen Blattart aus dem Nahrungsbrei im Darmkanal oft außerordentlich schwierig und langwierig ist, gibt die Darminhaltsuntersuchung bequem und zuverlässig Aufschluß über die Frage, ob eine Art vorwiegend oder nur teilweise als Blattzersetzer in Betracht kommt bzw. sich mehr oder weniger ausgeprägt von humosen Bodenbestandteilen o. a. ernährt. Der neuerdings erprobte Einsatz radioaktiver Isotope stellt eine weitere elegante, aber leider nicht fehlerfreie Methode zur Untersuchung der natürlichen Nahrungswahl dar.

Es ist weiter zu fragen, ob die Gültigkeit der in den eigenen Untersuchungen festgestellten Verhaltensweisen regional auf den Untersuchungsraum in Mitteldeutschland oder systematisch auf die untersuchten Diplopoden- und Isopoden-Arten beschränkt ist. Nach der vorliegenden Literatur ist beides zu verneinen. Soweit die mitgeteilten Untersuchungen auf fehlerfreien Beobachtungen auf vergleichbarer Basis beruhen, bestätigen sie durchweg die hier gegebene Darstellung der Verhältnisse. Die Schwankungen der Beurteilung verschiedener Autoren halten sich in den oben an einem Beispiel dargestellten Sicherungsgrenzen. Dies gilt wenigstens für Arbeiten aus den verschiedensten Teilen Europas. Der Vergleich mit den wenigen außereuropäischen Untersuchungen wird meist durch das Fehlen der für die Einschätzung nötigen chemisch-physikalischen Kenndaten des Nahrungsmaterials sehr erschwert. Schließlich geht aus eigenen Untersuchungen sowie aus der Literatur hervor, daß sich Lumbriciden, Enchytraeiden, Dipterenlarven, Gastropoden und sogar Amphipoden, die in den Tümpel gefallenes Laub befressen, hinsichtlich der Nahrungswahl so verhalten, wie es oben für Isopoden und Diplopoden beschrieben ist.

Hier mag die Frage angeschlossen werden, ob Mikrohumiphagen, wie z. B. Collembolen oder Milben, soweit sie wirklich von toter Pflanzensubstanz leben, in dieser Hinsicht eine andere Reaktionsnorm zeigen als für Makrohumiphagen dargestellt. Ein für die Bodenbildung höchst bedeutsamer Unterschied besteht darin, daß die Makrohumiphagen neben der Pflanzensubstanz Bodenpartikel aufnehmen, was Mikrohumiphagen nicht tun. Sieht man aber hiervon ab, so ist eine erstaunliche Übereinstimmung beider Bodentiergruppen in der Nahrungswahl beim Befraß von Fallaub festzustellen. Präferenzversuche mit fakultativ erstzersetzenden Collembolen, Folsomia fimetaria, ergaben im Vergleich zu Diplopoden und Isopoden eine durchweg gleichgerichtete durchschnittliche Präferenz. Auch diese Feststellung der gleichförmigen Reaktion bei der Nahrungswahl ist keine Einzelbeobachtung; besonders ist zu erwähnen, daß ein gleiches Nahrungswahlverhalten auch von Milben (Oribatiden) berichtet wird. Produktionsbiologisch und zur Klärung des Mechanismus der Nahrungswahl bedeutsam ist die Erscheinung, daß die gleiche Collembolenart als Zweitzersetzer die von den entsprechenden Blattnahrungen stammenden Kotballen der Diplopoden und Isopoden wiederum in gleicher Abstufung befrißt, ohne daß von der erstzersetzenden Tierart herrührende Unterschiede bemerkbar wären.

Im Vergleich zu der bei phytophagen Insekten weit verbreiteten Stenophagie ist die gleichförmig ausgerichtete Euryphagie der humiphagen Bodenarthropoden durchaus bemerkenswert. Bionomisch dürfte hierbei die oft ganzjährige Aktivität und die meist ohne ausgeprägte Larvenstadien verlaufende direkte Entwicklung der meisten hierzu gehörigen Tiergruppen im Gegensatz zu der in der Regel von Diapausen durchbrochenen metabolischen Entwicklung der meisten stenophagen Insekten eine Rolle spielen. Physiologisch mag bedeutsam sein, daß durch den beschriebenen Modus der Nahrungswahl in der Regel die Blattarten mit engem C/N-Verhältnis zuerst gefressen werden, die Arten mit weiterem C/N-Quotienten aber erst nach mehr oder weniger langer

Rottezeit, während der sich dieser Quotient wieder verengert. Es gibt aber nicht wenige Ausnahmen von dieser Regel, und eine Reihe weiterer Beobachtungen deuten darauf hin, daß mit der Berücksichtigung des C/N-Verhältnisses die physiologische Bedeutung der Nahrungswahl wenigstens nicht erschöpfend erfaßt wird. So konnte z. B. festgestellt werden, daß im allgemeinen weiche Blattarten mit hoher spezifischer Wasserkapazität, einem hohen N-Gehalt und geringer Huminsäurekonzentration bevorzugt wurden, wobei ein Einfluß des Calcium- und Aschegehaltes nicht nachweisbar war. Weiter zeigte es sich, daß dabei offensichtlich blatteigene oder aber von Mikroorganismen erzeugte Geschmacksstoffe eine bedeutende Rolle spielen. Ein besseres Verständnis der physiologischen Bedeutung dieser Vorgänge darf man sich von einer näheren Untersuchung der Mikroflora des Darmkanals und der sich im Darm abspielenden physiologischen Vorgänge versprechen.

Für produktionsbiologische Arbeiten ist die dargelegte Gleichförmigkeit der Nahrungswahl streuzehrender Humiphagen sehr wesentlich. Sie ermöglicht es, sich zur Ermittlung des Anteils der Fauna eines gegebenen Standortes an der Streuzersetzung auf die bedeutend einfachere und weniger zeitraubende Prüfung des Darminhaltes auf Fraß von Fallaub, Holz, Pilzen oder Bodenpartikeln zu beschränken. Inwieweit sich aus den geschilderten Befunden über den Spezialfall der Laubstreuzersetzung hinausgehende Folgerungen ziehen lassen, müssen weitere Untersuchungen klären.

DIE ABHÄNGIGKEIT DER VERMEHRUNG DES DERBRÜSSELKÄFERS (Bothynoderes punctiventris Germ.) VON DEN EIGENSCHAFTEN DES BODENS

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Der Derbrüsselkäfer ist in der Ukraine schon lange als ein sehr gefährlicher Zuckerrübenschädling bekannt. Seit mehreren Jahren studieren wir die Beziehungen zwischen der Massenvermehrung dieses Schädlings und den Bodeneigenschaften. Wir haben die Entwicklung des Rüsselkäfers in Beziehung zur Dynamik der physischen Bodeneigenschaften (Dichte, Porosität, Wasservorrat und Bodenluftgehalt) bei verschiedenen Bearbeitungsweisen erforscht.

Es wurde festgestellt, daß die Massenvermehrung des Rüsselkäfers durch lockere Bodenstruktur (staublehmige Tschernosjeme und Böden des Salzerdekomplexes) bedingt ist, weil hier die Larven in einem hohen Prozentsatz überleben, während sie an dichte Böden (tonreiche Tschernosjeme, schwere Lehme und podsolierte Böden) schlecht angepaßt sind. Das Kultivieren der Zuckerrübe auf lockeren Böden hat die Massenvermehrung des Schädlings hervorgerufen.

In den Böden mit lockerer Struktur haben die fußlosen, wenig beweglichen Rüsselkäferlarven im Gegensatz zu dichten Böden die Möglichkeit der freien Bewegung. Gleichzeitig ist in lockerer Erde auch die kräftige Entwicklung der Faserwurzeln der Pflanzen gesichert, von welchen sie sich nähren.

Als optimale Bodendichte für die Entwicklung der Larven erweist sich eine solche von 15—20 kg/cm² (Dichtemeßapparat unserer Konstruktion), bei einer höheren

Dichte des Bodens sinkt die Lebensdauer der Larven stark ab und es steigt die Vernichtungsrate durch Muscardinenpilze bedeutend an.

Bis jetzt dachte man, daß die normale Entwicklung der Zuckerrübe durch häufiges Auflockern des Bodens zwischen den Reihen begünstigt wird, dieses bewirkt aber gleichzeitig auch eine zunehmende Rüsselkäfervermehrung. Unsere Analyse der für die natürlichen Herde der Rüsselkäfervermehrung charakteristischen Bedingungen und die Verbreitungsareale der wildwachsenden Verwandten der Zuckerrübe zeigten, daß das Optimum der Bodenbedingungen für die Entwicklung des Rüsselkäfers und für das Wachstum der Zuckerrübe voneinander sehr verschieden sind. Der Rüsselkäfer ist in seiner Evolution mit lockerem Boden verbunden, die Zuckerrübe, dagegen mit Böden von dichter Struktur verknüpft.

Zahlreiche Laboratoriums- und Feldversuche weisen nach, daß durch Bodenbearbeitungsmaßnahmen, welche eine Zunahme der Dichte lockerer Böden bewirken, die Lebensfähigkeit des Rüsselkäfers auf ein Drittel oder Viertel herabgesetzt und die Zuckerrübenernte erhöht wird.

SYMPOSIUM X

INSEKTENLEBEN IN DER GROSS-STADT
(Großstadtbiologie)

DIE ENTWICKLUNG DER ARTHROPODENFAUNA IM STADTGEBIET VON HAMBURG

HERBERT WEIDNER, Hamburg

Wenn man die Tierwelt einer Großstadt mit den Augen des Biologen betrachtet, so kann man zahlreiche Probleme sehen, deren Bearbeitung nicht nur für die Praxis von Wert ist, sondern auch für die Erlangung von Erkenntnissen allgemeiner Art bedeutungsvoll werden kann. Ist doch die Großstadt wohl der vom Menschen am stärksten beeinflußte Lebensraum, den sich Tiere erobert haben. Am Beispiel Hamburgs möchte ich versuchen, die Entwicklung der Insektenfauna einer Stadtlandschaft in ihrer Abhängigkeit von der Entwicklung der Stadt selbst aufzuzeigen. Dabei werden wir auch am besten auf alle anderen einschlägigen Probleme stoßen, die uns eine Großstadtfauna stellt. Das gegebene Thema ist so umfangreich, daß ich hier nur auf die grundsätzlichen Fragen eingehen und Einzelheiten höchstens gelegentlich einmal als Beispiele anführen kann.

Die ursprüngliche, natürliche Fauna

Wollen wir die Veränderungen kennen lernen, die die Fauna einer Stadtlandschaft durch die Tätigkeit der Menschen erlitten hat, so müssen wir zunächst einmal versuchen, uns ein Bild von der Urlandschaft mit ihrer ursprünglichen Fauna in ihrer Abhängigkeit von Boden, Klima, geographischer Lage usw. zu machen. Geologie und Urlandschaftsforschung müssen uns dabei helfen. Der hamburgische Lebensraum hat seine Prägung durch die beiden letzten Eiszeiten erhalten. Die Endmoränenzüge der vorletzten Eiszeit, der Würm- oder Warthevergletscherung, erheben sich im Westen als ansehnliche Hügel (von 86 m bei Bahrenfeld und Blankenese über die 120 m hohen Harburger Berge bis zum 169 m hohen Wilseder Berg mitten in der Lüneburger Heide) über die flachen Grundmoränen der Riß- oder Saalevergletscherung. Im Nordosten flankieren die Landschaft die Endmoränen der Weichselvergletschung (von den Wartenbergen bei Ahrensburg über die Höhen des Bocks- und Schüberges bis hin nach Tangstedt). Während die Moränenzüge der Weichselvergletscherung das Landschaftsbild wenig beeinflußt haben, räumten ihre Schmelzwasser die Urelbe zum Urstromtal aus. Gleichzeitig zog der neugebildete Strom die Entwässerung des böhmisch-sächsischen Raumes an sich, der zuvor durch das Ohre-Aller-Urstromtal entwässert wurde. Nach dem Verlaufen der Gletscherwasser war das weite Urstromtal der Elbe ein Stromverwilderungsgebiet mit vielen Wasserarmen und dazwischenliegenden Talsandflächen. Das Klima war zunächst noch rauh und windreich. Die Vegetation war spärlich. Die Flußsande wurden vom Wind im Westen auf die Grundmoräne bei Holm und Wedel hinaufgeweht, während sie im Osten bei Boberg sich an dem Steilrand des Urstromtales stauten und im Elbtal liegen blieben. Bis heute haben sich diese Dünen in den Holmer Sandbergen noch gut erhalten, während sie bei Boberg erst in der Nachkriegszeit weit-

gehend vernichtet wurden. Die offenen und warmen Dünenböden sind besonders bei Boberg von einer reichen und eigenartigen Tierwelt besiedelt worden, die aus dem Osten durch das Elbtal zugewandert ist. Auch der Mensch hat sich schon frühzeitig auf ihnen niedergelassen, wie die vielen Kulturspuren aus der Alt- und Jungsteinzeit zeigen.

Die Vegetation hat in der postglazialen Zeit bis zur Zeit der Geschichtsschreibung noch manche Wandlungen durchgemacht, über die wir durch die qualitative und quantitative Untersuchung des in den Torfen und in den Schlammablagerungen der Gewässer enthaltenen Blütenstaubes der Waldbäume sehr gut unterrichtet sind. Nach Abschmelzen des Eises herrschte noch ein arktisches und subarktisches Klima. Polarweiden und Zwergbirken bildeten die ersten Ansiedler. Ihnen folgten geschlossene Birkenwälder, zu denen sich Kiefern gesellten, die infolge eines Kälterückschlages sich eine Zeitlang wieder zurückzogen, um bei zunehmender Wärme und Trockenheit die Vorherrschaft anzutreten. Mit Zunahme der Niederschläge wanderten Eiche, Ulme, Linde und Erle ein. Ein stark differenziertes Waldbild bildete sich in Abhängigkeit von der Bodenbeschaffenheit aus. Hainbuche und zuletzt die Rotbuche kamen noch dazu. Zwischen 6000 und 5000 vor Christi Geburt begann infolge eines Wasserrückstaus die Talversumpfung, die durch die Erlenbruchwälder weiter gefördert wurde. Die alten Bruchwälder gingen allmählich zugrunde. Flachmoore und später Hochmoore bildeten sich an ihrer Stelle. Erst gegen Ende der Bronzezeit, etwa in der 2. Hälfte des 1. Jahrtausends vor Christi Geburt, griff der Mensch verändernd in das Landschaftsbild ein. Er brauchte Raum für Getreideanbau. Doch waren diese Eingriffe noch gering. Sie wurden erst im eigentlichen Rodungszeitalter vom 13.—16. Jahrhundert nach Christi Geburt einschneidend und für weite Gebiete umgestaltend.

In der Litorinazeit, etwa um 1600 vor Christi Geburt, brach das Meer in das Elbtal ein und drang bis in die Gegend der heutigen Vierlande vor. Damit begannen die durch Ebbe und Flut bedingten regelmäßigen Schlammablagerungen in der Gezeitenzone, die Marschenbildung. Die Marschschicht ist bei Hamburg noch von geringer Mächtigkeit, wird aber zur Elbmündung immer dicker; denn je länger ein Gebiet der Überflutung ausgesetzt ist, um so höher schlickt es aus. Je nachdem, ob die Sedimente vom Meer oder vom Fluß abgesetzt werden, unterscheidet man zwischen Meeres- und Flußmarsch. Die Flußmarschbildung hält auch jetzt noch im Hamburger Gebiet an. Im Lauf der Jahrhunderte tauchten aus der Elbe die Inseln auf, die auf ihrem fruchtbaren Boden Bruchwälder trugen. Mit der Marschenbildung hat sich der typische Gegensatz des hamburgischen Landschaftsbildes ausgeprägt: Geest und Marsch, der sich auch auf die Insekten ausgewirkt hat, so z. B. durch Entstehung von in ihren erblich fixierten Schlüpfzeiten verschiedenen Marsch- und Geestrassen von *Cheimatobia brumata* L. (Speyer 1939, S. 2421).

Für die Tierwelt und besonders für die Insektenwelt dieser Urlandschaft haben wir wenige direkte Zeugen. Es fanden sich allerdings in den postglazialen Ablagerungen verschiedene Insektenreste, die man einigermaßen gut datieren kann, doch sind die so gefundenen Arten nicht sehr zahlreich und stellen im wesentlichen auch nur eine Auswahl von Wasserinsekten oder wenigstens in Wassernähe lebender Insekten dar. Von Pflanzenschädlingen wissen wir, daß in der Buchenzeit die Gallmilbe *Eriophyes laevis* Nal. an Erle und die Gallwespen *Neuroterus laeviusculus* Schenck und *N. quercusbaccarum* L. an Eiche ihre Gallen bildeten. Auch der Maikäfer, *Melolontha melolontha* L. hat bereits im ursprünglichen Wald der Buchenzeit Bäume entlaubt. Vor 1000 Jahren kam auch schon die Gallwespe *Cynips divisa* Hartig vor (Beyle).

Da viele Insekten in ihrer Lebensweise an bestimmte Pflanzenarten gebunden sind, so können wir aus der gegenwärtigen Verbreitung der Insekten und aus der Kenntnis der Flora jener fernen Zeiten mit ziemlicher Sicherheit auf die Zusammensetzung der

Insektenfauna schließen. Diese Insekten sind den Klima- und Vegetationsschwankungen der postglazialen Zeit folgend zu- und abgewandert, wobei manche kälteliebende Arten sich auf Kälteinseln wie Mooren, in dem wärmer werdenden Gebiet bis auf die Gegenwart als „Eiszeitrelikte" herrüberretten konnten. Die Wanderwege, auf denen die Insekten aus ihren Eiszeitrefugien unser Gebiet besiedelt haben, lassen sich für viele Arten mit den Methoden der historischen Tiergeographie rekonstruieren. Die einen Arten kamen aus dem sibirisch-amurischen Waldgebiet teils über Finnland und Schweden, teils an der Südküste der Ostsee entlang, andere aus den pontischen Steppengebieten teilweise unter Benutzung der Urstromtäler und wieder andere aus dem mediterranen Waldgebiet über Spanien und das damals noch zum Festland gehörende England.

Diese Ausweitung des Areals kann für viele Tierarten auch heute noch fortgehen, ohne irgendwie vom Menschen erkennbar, beeinflußt zu werden. Gewöhnlich sind die Insekten abhängig von der allmählichen Ausbreitung ihrer Futterpflanzen. So entstand die Urlandschaft mit ihrer eigentümlichen Insektenfauna. Sie bildet die Grundlage, auf der die Menschen die Kulturlandschaft aufzubauen begannen, in der sich aber überall wo es möglich war, auch noch Reste der ursprünglichen Fauna erhalten haben. So hält z. B. Lohse 13 von 26 Carabiden-Arten des mitten in Hamburg gelegenen Botanischen Gartens für Arten des unbebauten, natürlichen Geländes. Sie zu kennzeichnen ist nicht immer leicht. Jede Art muß besonders daraufhin untersucht werden. Andere Bestandteile der ursprünglichen Fauna sind dagegen für die Einwirkung des Menschen außerordentlich empfindlich. Sie verschwinden bereits, wenn der Mensch in ihre Nähe kommt und ihr Lebensraum nach unserem Dafürhalten eigentlich noch gar nicht verändert ist. Bekannte Beispiele hierfür aus dem Hamburger Faunengebiet sind die beiden Bärenspinner *Pericallia matronula* L. und *Arctia hebe* L.

Die Fauna der Kultur- (Agrar- und Forst-)Landschaft

Wie wir bereits gehört haben, begannen die Menschen, die schon in der Steinzeit, vor rund 15.000 Jahren, den Rentierherden folgend, im Alster- und Billetal in den kurzen Sommern vorübergehend ihre Zelte aufgestellt hatten, in der Bronzezeit sich dauernd anzusiedeln und Getreide anzupflanzen. Damit dürften sie schon große Veränderungen im Faunengebiet herbeigeführt haben. Die Insekten reagieren nämlich sehr fein auf die geringste Veränderung einer Landschaft. Dies ist ein Punkt, der bei allen kultur-zoologischen Betrachtungen stärkste Beachtung verdient. In Hamburg hat O. Kröber dieses einmal sehr schön an der Dipterenfauna des unmittelbar am Stadtrand liegenden Eppendorfer Moores gezeigt, das er fast ein halbes Jahrhundert planmäßig besammelt hat. Sein Hauptuntersuchungsgebiet war eine im Moor gelegene Heidepartie. 1909 wurde das Moor zum Volkspark erklärt und große Teile anlageartig hergerichtet. Die Heidepartie aber blieb sich vollkommen selbst überlassen. Durch die Änderung ihrer Umgebung hat sie aber ihren Charakter ebenfalls geändert, und sie wuchs zu einem Birkenwäldchen aus, wodurch die lichtliebenden Bodenpflanzen vertrieben wurden. Die Änderung in der Fliegenfauna innerhalb der 24 Jahre ist erstaunlich: Von den vor 1909 festgestellten 535 Arten und den nach 1933 aufgefundenen 841 Arten waren nur 200 noch gleich. Von ersteren sind also 335 Arten verschwunden und von letzteren 641 dazugekommen. Kröber urteilt selbst über sein Sammelergebnis: „Interessant für den Entomologen ist wohl weniger die große Dipterenzahl, die aus diesem kleinen Stückchen Moorgelände herausgeholt wurde, als vielmehr der auffallende Unterschied zwischen der Besiedlung vor der Waldwerdung und nachher. Daß heute viel mehr Arten vom Moor vorliegen als bei Abfassung meiner ersten Fauna, mag z. T. begründet sein in der Anwendung anderer Sammelmethoden, die namentlich Kleintiere ins Netz bringen, z. T. in intensiverer Sammeltätigkeit. Aber daß eine ganze Anzahl Arten, ja ganze Familien, seit 1933 nicht wieder trotz eifrigen Suchens auf-

gefunden wurden, daß Familien, von denen vor 1909 nie ein Exemplar erbeutet wurde, jetzt in vielen Arten vorliegen, das spricht doch sicher für eine vollständige Umänderung der Lebensbedingungen in dieser Zeitspanne.“ Die Tatsache, daß eine Landschaft durch Veränderung ihrer Umgebung selbst einen anderen Charakter erhält, ist eine Gefahr, die bei der Erklärung von Naturschutzgebieten, besonders wenn sie im Weichbild einer Großstadt liegen, sehr leicht von den zuständigen, oft nicht genügend biologisch vorgebildeten maßgebenden Stellen übersehen wird. Diese Gefahr ist natürlich um so größer, je kleiner das unter Naturschutz gestellte Gebiet ist. Diese Bemerkungen nur nebenbei als praktisches Ergebnis der rein wissenschaftlichen Forschungsarbeit.

Noch tiefgreifender wurde der Einfluß des Menschen auf die ursprüngliche Landschaft und Fauna in der eigentlichen Rodungszeit, vom 13. bis 16. Jahrhundert. Der Wald wurde entfernt. Man brauchte Platz für Siedlungen und Felder. Es wurde auch ohne Rücksicht auf die Zukunft durch Holzentnahme und Vieheintrieb ausgebeutet. Weiden und Felder, Gärten und Forste wurden angelegt Die Wasserläufe wurden durch Deiche in ein enges Bett gezwängt und ihnen dadurch Land abgewonnen, das sofort wirtschaftlich genutzt wurde. Andererseits wurden große Mühlenteiche, wie die Alster, ein künstlicher See, aufgestaut. Vor den Toren der Stadt Hamburg entstanden die großen Gartenlandschaften der Vierlande und des Alten Landes und viel später das Baumschulengebiet von Rellingen, von denen jede ihr ganz besonderes Gepräge hat. Die Moore wurden entwässert, der Torf abgestochen usw. Durch alle diese Maßnahmen wurde auch das Klima weitgehend beeinflußt und ganz besonders die Tierwelt. Vielen Tieren wurde in weiten Gebieten der Lebensraum genommen und sie damit zum Aussterben verdammt. Andere fristen nur in kleinen Rückzugsgebieten, die auch oft erst vom Menschen wieder neu angelegt wurden, wie die Wallhecken oder Knicks Schleswig-Holsteins, ihr Dasein. Wieder anderen Arten dagegen wurde der Tisch durch Anpflanzen von Kulturpflanzen reichlich gedeckt, so daß sie sich in Massen vermehren konnten. Außerdem hat aber der Mensch noch vielen Arten Lebensraum geboten, die früher nicht in dem Gebiet heimisch waren, da ihre Futterpflanzen fehlten. Im allgemeinen kann man die Faustregel aufstellen, daß, wenn Kulturpflanzen bei uns im Freien gedeihen, ihnen auch ihre Schädlinge folgen können. Sie werden mit den Kulturpflanzen eingeschleppt oder folgen ihnen durch aktive Wanderungen. Wir vergessen gar zu leicht, daß viele unserer heute häufigen Freilandinsekten ihr Dasein nur der menschlichen Tätigkeit verdanken. Sind doch Fichte und Lärche, heute Hauptbestandteile unserer Wälder im Hamburger Gebiet, nördlich der Elbe erst in der Mitte des 18. Jahrhunderts aufgeforstet worden. Vorher konnte es heute so häufige Insekten wie *Adelges laricis* Vall., *Sacchiphantes viridis* Ratz. und *Coleophora laricella* L., um nur einige zu nennen, in unserem Gebiet nicht geben. Der Anbau oder die Aufforstung gebietsfremder Pflanzen haben nicht nur neue Schädlinge gebracht, sondern oft auch den bereits vorhandenen Tieren bessere Lebensbedingungen geboten, so daß sie auf die gebietsfremden Pflanzen übergegangen sind. Auch dafür gibt es viele Beispiele in unserem Gebiet (z. B. Schmetterlinge, die Loibl, S. 40, zusammengestellt hat).

So schiebt sich also über die Schicht der ursprünglichen Fauna eine zweite Schicht von Tieren, die mit den Kulturpflanzen in das Gebiet eingedrungen sind, bzw. die aus der Urlandschaft in die Agrar- und Forstlandschaft übergegangen sind, dort also nicht nur als Reste ihr Leben kümmerlich fristen, sondern zu vorherrschenden Gliedern der Fauna der Kulturlandschaft geworden sind. Die dort herrschenden ökologischen Verhältnisse hat uns Tischler in Schleswig-Holstein in seinen agrar-ökologischen Untersuchungen aufgedeckt. In dem Hamburger Landgebiet nördlich der Elbe werden ähnliche Verhältnisse herrschen. In den oben genannten Garten- und Baumschulgebieten müßten sie allerdings noch eingehender untersucht werden.

Die Fauna der dörflichen Siedlungen

Wie sich die Fauna der Kulturlandschaft über die Urlandschaft schiebt, so legt sich jetzt auf die der Kulturlandschaft eine ebenfalls vom Menschen geschaffene Fauna, die der Siedlungen. Wir haben zwei Typen von Siedlungen zu unterscheiden, die ländliche, offene Siedlung und die Stadt. Bei der ersteren werden ein Hof oder einige wenige Gehöfte in die Kulturlandschaft hineingestellt. Haus und Hof mit Viehställen und Vorratshäusern bilden einen kleinen Lebensraum für sich, in dem Tiere ein Lebensoptimum finden durch die mikroklimatischen Bedingungen im Haus und durch die Anhäufung ihrer Vorzugsnahrung, die sie im Freien in dieser Menge nicht finden. Ursprüngliche Bewohner von Abfall, Müll und Nestern ziehen sich in diesen vom Menschen neugeschaffenen Lebensraum. Vögel (Tauben, Schwalben), Fledermäuse und Nager folgen, und mit ihnen kommen ihre Parasiten und Nestbewohner. Auch das zum Bau des Hauses verwendete Holz, das zum Decken des Daches verwendete Rohr, ja selbst das zunächst aus Lehm und Mörtel mit Ziegeln bestehende Baumaterial für die Wände wird von allerlei Insekten (z. B. *Colletes daviesanus* SM.) aus der umgebenden Landschaft besiedelt, die sich dann in einem natürlicherweise nicht möglichen Ausmaß vermehren. In Hausnähe wird ein Garten angelegt. Besondere Kulturpflanzen werden kultiviert, die aus fern entlegenen Gebieten herbeigeschafft werden. Besonders im Garten erfolgt so auf engem Raum eine Häufung neuer Lebensräume. Durch Handel und gegenseitige Besuche werden langsam aber sicher immer mehr Tiere von Siedlung zu Siedlung verschleppt. Es bildet sich so allmählich eine typische, der Siedlung eigentümliche Fauna heraus, deren Vertreter zum Teil der Fauna der Ur- oder Kulturlandschaft entstammen, dort selbst aber mehr oder weniger vollständig verschwunden sind, zum Teil aber fremden Faunen angehören. Wir können hier ein gleitendes Übergehen der drei Faunenkomponenten ineinander verfolgen.

Die Fauna der ummauerten Stadt

Anders ist die Entstehung der Fauna der mittelalterlichen Stadt. Zwar mögen die ersten Anfänge zunächst ebenso wie bei den geschilderten ländlichen Siedlungen gewesen sein. Sobald aber ein Ort mit einer Mauer umgeben wurde, wurde der Raum innerhalb der Mauer als Vorrats- und Wohnplatz für Mensch und Vieh hergerichtet. Er wurde eng mit Häusern und Ställen zugebaut, für Vegetation war kein Raum in ihm. Die Gärten lagen vor der Mauer. Für Hamburg mag dieser Zustand um 1300 erreicht gewesen sein. Mit dem Zusammenpferchen der Menschen und des Viehs entstanden ganz neue Probleme, mit denen die Menschen jahrhundertelang nicht fertig geworden sind. Dies war die Beseitigung der Abfallstoffe des Lebens. Müll, Kot, Küchenabfälle, Abwasser standen in der Stadt, die Straßen bildeten einen einzigen Morast, in dem sich die Schweine suhlten. Ein pestialischer Gestank herrschte in den Gassen, die man nur reitend oder in Sänften getragen passieren konnte, wenn man nicht bis zum Knöchel oder noch tiefer im Schmutz versinken wollte. Selbst die Kadaver toter Tiere lagen auf der Straße, bis sie von Insekten, besonders Fliegenmaden, beseitigt worden waren. Die Fliegenplage in einer mittelalterlichen Stadt muß unvorstellbar gewesen sein. Kein Wunder, wenn immer wieder neue Seuchen auftraten und Tausende als Todesopfer forderten, obwohl sich gegen endemische Dysenterie eine Art Immunität entwickelt haben müßte (Gernet). Erst gegen Ende des 18. Jahrhunderts stellen wir Bestrebungen fest, diesen Mißständen ein Ende zu machen. Sie fallen zeitlich etwa zusammen mit der Beseitigung der Stadtmauern, so daß man den ganzen Zustand der Stadt als „mauerumgebene Stadt" im Gegensatz zu der gleichzeitigen dörflichen Siedlung und der späteren „offenen Stadt" bezeichnen könnte.

Über die Tierwelt der ummauerten Stadt wissen wir wenig. Nur aus gelegentlichen Angaben aus alter Zeit können wir uns ein Bild davon machen bzw. es aus den damals

herrschenden ökologischen Verhältnissen uns rekonstruieren. Die Fauna der ummauerten Stadt wird zunächst die typischen Tierarten der dörflichen Siedlung ebenfalls zum großen Teil enthalten haben. Allerdings ohne die Relikte aus der Ur- und Agrarlandschaft. Die Entwicklung der Fliegen und anderer in Abfallstoffen sich entwickelnden Insekten muß sich gesteigert haben. Selbst aus den in den Kirchen der Stadt begrabenen Leichen entwickelten sich Scharen charakteristischer Insekten (*Conicera tibialis* Schmitz) (Buchner S. 91). Die eng gebauten Häuser ließen kaum einen Sonnenstrahl in die Höfe, geschweige denn in die niedrigen Zimmer dringen. Feuchtigkeit und dumpfe Wärme herrschten im Sommer, Kälte, die nur durch geringe Heizmöglichkeiten kaum gemildert werden konnte, im Winter. Feuchtigkeitsliebende Insekten, z. B. die Pelzmotte, die Mehl- und Fettzünsler, die Kornmotte und Flöhe dürften in den Häusern gelebt haben, während Kleidermotte, Bettwanzen und andere die Wärme und Trockenheit liebende Hausinsekten selten waren oder ganz gefehlt haben. Aber auch schon in der mittelalterlichen Stadt herrschte die Sucht nach einem besseren Leben, und deshalb wurden die verschiedensten Nahrungs- und Genußmittel und Güter aller Art aus fremden Ländern herbeigeschafft. Wenn auch die Handelsbeziehungen noch nicht weit reichten, so wurden auch schon durch diesen beschränkten Handel Insekten eingeschleppt, in einer Handels- und Hafenstadt wie Hamburg in weit höherem Grade als in einem anderen, binnenländischen Ort. Aber auch über diese Insekten, die schon damals die Handelsware verdorben haben müssen, hören wir nur selten einmal etwas. Man war an die Insekten gewöhnt und machte kein Aufsehen davon. Die Assekuranz- und Havarieordnung von 1731 befreite ausdrücklich den Versicherer von der Haftpflicht für inneren Verderb der Ware infolge ihrer natürlichen Beschaffenheit, z. B. wenn „Kastanien und Korn sich anstecken“ (Kiesselbach S. 145).

Während in der ummauerten Stadt selbst kaum Platz für Freilandinsekten war, so boten sich ihnen in den Gärten vor den Mauern eine Vielzahl verschiedener Lebensräume, wahrscheinlich in größerer Zahl als in den Bauerngärten, weil durch die Gartenliebhaberei der reichen Stadtbewohner eine große Zahl ausländischer Pflanzen herbeigeschafft und angepflanzt wurde. Mit ihnen müssen auch manche fremdländische Insekten gekommen sein. Aber auch hierüber wissen wir wenig Genaues. Linné schreibt in seinem Tagebuch über seinen Besuch in Hamburg (28. 4. bis 16. 5. 1735): „In den Gärten gab es ein Insekt, bunt, mit Spargeln aus Rußland gekommen, qui devastat Asparagus.“ Es war *Crioceris asparagi* L. (Bryk, S. 200).

Die Fauna der offenen Stadt

Erst im 18. Jahrhundert beginnt man auf Grund der fortgeschrittenen hygienischen Erfahrungen die ökologischen Verhältnisse in der Stadt zu ändern und erreicht damit ganz ungewollt auch eine Beseitigung mancher Insektenplagen. Auch schon Bekämpfungsbestrebungen von schädlichen Insekten werden deutlicher und einige fortschrittliche Männer befassen sich ernstlich mit ihrer Erforschung. 1737 verkaufte in Hamburg Georg Friederich Quantz in der Knochenhauer-Straße „(sonst aber nirgends)“ D. Southalls in England hochgepriesene „Wantzen-Mixtur“ und dessen Bericht darüber in deutscher Übersetzung. Das Glas dieser Mixtur zu 4 Loth kostete 12 und der Bericht 2 Schilling. Die Beschäftigung mit den Insekten wird eine gern geübte Liebhaberei und Wissenschaft. Als erster befaßte sich mit der Fauna Hamburgs Johann Georg Lehmann, der seit 1820 im Akademischen Gymnasium „historiam naturalem animalium, quae Faunam Hamburgicam spectant“, las. Zunächst registriert man nur das Vorkommen der Insektenarten, dann beachtet man die Verschiedenheiten ihrer Fundorte. Solche Faunenlisten wurden in Hamburg in den letzten hundert Jahren fast von allen Ordnungen ausgearbeitet, von manchen schon in mehreren Auflagen. Aus ihnen geht hervor, daß sich die Insektenfauna auch innerhalb des Stadtgebietes sehr geändert hat.

Nach dem Fallen der Mauern hat sich die Stadt rasch ausgedehnt. Die alte ummauerte Stadt ist zur Innenstadt geworden, die ländlichen Siedlungen in ihrer Umgebung sind immer mehr von ihr aufgesogen worden. Wo vor 50 Jahren noch ergiebige Fangplätze für den Entomologen waren, stehen jetzt Häuser. Aus den Außenbezirken der Stadt kommen nun ganz andere Faunenelemente in sie hinein. Um die Innenstadt zieht sich ein Ring von aufgelockert gebauten Stadtteilen. In Hamburg wird das Häusermeer, das ökologisch betrachtet einer Wüste gleicht, weshalb ich es Kulturwüste genannt habe, vielfach unterbrochen von großen Parks und vom Wasserbecken der Alster. Sie haben eine eigene Fauna mit Relikten aus der Urlandschaft, aber auch mit eingeschleppten Arten und Zuwanderern. Einheimische Arten sind auf fremdländische Nährpflanzen übergegangen. Vom Stadtrand, über die allee- und gartenreichen Vorstädte bis zur Innenstadt, lassen sich Zonen einer allmählichen Faunenveränderung feststellen. Freilich sind die Übergangszonen sehr verwaschen und unbeständig.

Wie vom Stadtrand her die Freilandinsekten nach der Innenstadt zu allmählich abnehmen, so strahlen vom Hafengebiet her eine Unzahl fremdländischer, durch die Schiffe ständig eingeschleppter Insekten, zum großen Teil Vorrats- und Materialschädlinge, aus, deren Bedeutung man erst vor 100 Jahren allmählich zu begreifen begonnen hat. Die erste Erkenntnis, die man staunend gewann, war, daß sich Insekten aus Übersee auch bei uns unter besonderen Bedingungen entwickeln können (Augustin). Durch die Gefahr, daß die als großer Pflanzenschädling gefürchtete San José-Schildlaus aus Amerika auch nach Europa eingeschleppt werden könnte, wurde 1898 im Hamburger Hafen die „Station für Pflanzenschutz“ gegründet, deren Beamte auch auf andere eingeschleppte Tiere achteten. Als Ergebnis ihrer Sammeltätigkeit veröffentlichte 1901 K. Kraepelin eine Arbeit „Über die durch Schiffsverkehr in Hamburg eingeschleppten Tiere“. In dieser Liste stehen regelmäßig eingeschleppte Vorratsschädlinge und durch Zufall einmalig zu uns gelangte Tiere nebeneinander, ohne daß es dem Verfasser möglich gewesen wäre, ein Werturteil über die Bedeutung dieser Tiere zu fällen. Diese Liste ließ aber die Zoologen in Deutschland auf die eingeschleppten Tiere aufmerken und lenkte sie auf die Bearbeitung ihrer Lebensweise hin. Uns hat zunächst einmal die Frage nach den Einbürgerungsmöglichkeiten für die einzelnen Arten zu interessieren. Dazu sind in erster Linie autökologische Untersuchungen nötig, auf die synökologische folgen müssen. Dabei ist zu beachten, daß sich in der Großstadt durch die Häuser, die die Wärme speichern, sowie durch eine Dunstkappe, die sich über ihr bildet, ein ausgeglicheneres Klima mit höheren Temperaturen und in Hamburg auch mit stärkeren Niederschlagsmengen als in der Umgebung (van Eimern) ausbildet. Manche Insektenarten können sich schon dadurch im Stadtgebiet halten, für die es außerhalb zu kalt sein würde. Dazu kommt dann noch, daß durch die Tätigkeit des Menschen die durch das Klima normalerweise gefährdeten Entwicklungsstadien von Insekten geschützt werden, wie dies z. B. bei *Acanthoscelides obtectus* Say der Fall ist. Sein Freilandauftreten in Hamburg war nur möglich, weil verkäferte Bohnen alljährlich vom Menschen gesteckt und die in den reifen Bohnen lebenden Larven durch die Aufbewahrung der Saatbohnen in frostfreien Räumen vor der Vernichtung durch die winterliche Kälte bewahrt wurden. Von einer Anpassung des Insekts an ein kälteres Klima kann in einem solchen Fall keine Rede sein (Zachariae). Viele vorgefaßte Meinungen müssen in dieser Hinsicht wohl noch revidiert werden.

Von großem Einfluß auf die Insektenfauna der Stadt sind die Veränderungen, die die fortschreitende Technik mit sich bringt. Änderungen im Baumaterial (erst nach Ablösung der Eiche als Bauholz durch Kiefer und Fichte erlangte *Hylotrupes bajulus* L. seine große Bedeutung, während die von *Xestobium rufovillosum* Deg. fast vollkommen auf Null gesunken ist), in der Heizung, Ersetzen der Pferdefuhrwerke durch Motorfahrzeuge, die Verwendung von Kunststoffen usw. verändern auch die Insektenwelt.

Dazu kommen schließlich noch die immer besseren Bekämpfungs- und Vorbeugemethoden.

Eine weitere Frage, die sich hier erhebt, ist, ob durch den Einfluß des Menschen die Insekten verändert werden. Veränderungen des Verhaltens der Insekten im Stadtgebiet wurden an vielen Arten beobachtet. Es gehören hierher alle die Arten, die sich neue Futterpflanzen gesucht haben. Änderungen im Aussehen von Stadtinsekten haben gerade in Hamburg schon frühzeitig das Interesse der Forscher erweckt, und zwar in der Erscheinung, die man als neuzeitlichen Großstadt- oder Industriemelanismus bezeichnet hat. In den ersten Jahren dieses Jahrhunderts sind melanistische Mutationen bei Schmetterlingsarten aufgetreten, die bald die Stammform fast völlig verdrängt haben. Man glaubte sie als ein Produkt der Industrieabgase ansehen zu müssen. Hasebroek hat in Hamburg deshalb mit mehr oder weniger gutem Erfolg viele Versuche gemacht. Ganz geklärt ist die Erscheinung aber bis heute noch nicht. Eine andere Form der Veränderungen der Insekten durch den Menschen ist das Auftreten von insektizidfesten Stämmen nach großen Bekämpfungsaktionen. Das Studium aller dieser Erscheinungen ist wichtig für die Erklärung der Entstehung der eigentlichen Hausinsekten, der Arten, die jetzt so gut wie ausschließlich nur noch im Haus bzw. in vom Menschen geschaffenen Lebensräumen auftreten können. Eng damit zusammen hängt auch die Erscheinung der Verstädterung der Vögel (Amseln, Möwen, Eichelhäher, Krähen usw.), die eine Verhaltensänderung darstellt. Durch die Nistplatzverlegung dieser Vögel in die Stadt, erhält deren Fauna Zuwachs durch nidicole Insekten und Milben. Über eine ökologische Gliederung des Stadtgebietes von Hamburg habe ich bereits auf dem Internationalen Entomologenkongreß in Berlin 1938 berichtet. Seitdem konnten einige Lebensräume näher untersucht werden. Wenn dabei nicht nur auf Schädlinge geachtet wird, so kann man oft recht überraschende Ergebnisse erzielen. So brachte z. B. die Untersuchung eines Müllplatzes die Feststellung von 26 Spinnenarten, von denen in Deutschland einige als selten galten und 1 Art (*Ostearius melanopygius* Cambridge) bisher überhaupt noch nicht festgestellt worden war (Braun).

Einer Erörterung bedarf dabei auch die Frage, ob wir es in der Großstadt wirklich mit echten Biotopen zu tun haben. Diese Frage ist vielfach verneint worden. M. E. muß sie bejaht werden, wenn man den Menschen als wichtigsten ökologischen Faktor nicht außer acht läßt. Freilich sind in der Stadt die einzelnen Bestandteile der Biotopmosaiks vielfach kleinräumiger als in der freien Natur und daher der völligen Vernichtung leichter ausgesetzt.

Die Fauna der offenen Stadt hat ebenfalls in den letzten 150 Jahren eine weitere Entwicklung genommen, die auch jetzt noch anhält. In Hamburg, wie in vielen anderen europäischen Großstädten, brachte der letzte Krieg ein böses Zwischenspiel. Große Stadtteile wurden ein Raum der Flammen. Wo das Feuermeer wütete, konnte kein Lebewesen weiter existieren. An Stelle der Häuser blieben Trümmerstätten übrig, die rasch von Pflanzen und Tieren neu besiedelt wurden. Das früher nur von Waldschlägen bekannte *Epilobium*, das man in Hamburg die Trümmerrose nannte, war eine der häufigsten Pflanzen auf den Schuttfeldern. Und schon traten Insekten, die wie die Weinschwärmer auf ihm leben, ebenfalls häufig in der Stadt auf. Leider wurde die Trümmerfauna in Hamburg mit Ausnahme von *Anopheles maculipennis* Meigen (Heinz) nicht eingehend untersucht. Es dürften dort aber ähnliche Verhältnisse wie in anderen Städten, z. B. Braunschweig, geherrscht haben.

Jetzt sind die Wunden des Krieges wieder beseitigt. Neue Gebäude oder Anlagen sind auf ihnen entstanden. Dazu kommt noch, daß Hamburg von einer Großstadt zu einer Weltstadt geworden ist, ein geographischer Begriff, der erst in den letzten Jahren geschaffen wurde. Eine Weltstadt haben wir vor uns, wenn mehrere, ziemlich selbständige Städte zu einem Ganzen verschmolzen sind, mit ländlichen Zwischen-

gebieten, mit besonders starkem Verkehr mit der ganzen Welt usw. Es bildet sich ein neues geographisches Landschaftsbild heraus. Zu den Geschäfts- und Wohnvierteln kommen noch die notwendigen großen Erholungsgebiete dazu. Mir scheint, daß sich auch hier eine neue Faunenzusammensetzung ausbildet, wenigstens deuten manche neue Schädlingsprobleme darauf hin, wie z. B. das in den letzten Jahren gehäuft beobachtete, von der modernen mit Rasenflächen durchsetzten, aufgelockerten Bauweise abhängige Massenauftreten von *Bryobia* (Rack), wie die Taubenplage mit ihren Insektengefolgschaften usw. Nicht vernachlässigen darf man dabei auch die naturfremde Einstellung des Weltstädters der Tierwelt gegenüber, die sich einerseits in zu großer Ungezieferscheu zeigt, die in Ungezieferwahn ausarten kann, andererseits aber auch in einer übergroßen Gefühlsduselei dem Tier gegenüber.

Die Ziele aller großstadt-entomologischen Untersuchungen sind zweierlei Art. 1. sollen an dem großartigen Experiment der Großstadtinsekten allgemeine Gesetzmäßigkeiten aufgefunden werden und Fragen, wie die Veränderung der Insekten durch ihre Umwelt, die Besiedlung tierleerer Gebiete, die Einbürgerungsmöglichkeiten fremder Tiere usw., beleuchtet werden. 2. soll aber auch durch die Kenntnisse der Ökologie der schädlichen Insekten ihrer Einschleppung, Ansiedlung oder Massenvermehrung entgegengearbeitet werden. In jeder Stadt liegen die Verhältnisse etwas anders, bedingt durch ihre geographischen Gegebenheiten. Daher müssen überall diese Verhältnisse untersucht werden, erst dann werden die allgemein für die Großstadt geltenden Gesetzmäßigkeiten von den lokal bedingten Voraussetzungen zu unterscheiden sein. Erst dann werden wir wirklich von einer Großstadtentomologie bzw., weiter gefaßt, von einer Großstadtbiologie sprechen können.

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DISKUSSION

B. HEYDEMANN: Ist es im Falle der Stadt Hamburg so, daß der Stadtkomplex die Niederschlagsmenge positiv beeinflußt, oder liegt die Stadt — auf Grund der Niederschlagskarte von Hamburg und Umgebung — zufällig in einer Ecke höheren Niederschlags im Süden von Schleswig-Holstein? Besonders wesentlich erscheint mir bezüglich des Niederschlagsfaktors im Großstadtklima auch die Art des Niederschlags, da z. B. eine weitaus geringere Menge des jährlichen Niederschlags in der Großstadt als Schneelage in Erscheinung tritt als außerhalb dieser, Schnee aber sehr viel mehr als Regen die jährliche Aktivitätsperiodik der Bodenarthropoden beeinflußt.

H. WEIDNER: Der Stadtkomplex beeinflußt die Niederschlagsmenge positiv. Darüber gibt es mehrere Arbeiten von Meteorologen für Hamburg und andere Städte.

L. P. LEFKOVITCH: I think that the most interesting studies will be in the comparison between the fauna of cliffs and walls, caves and cellars, and gardens and fields; including the reasons for the absence or presence of species which would be present in the comparable environment.

H. KEMPER: Von einer Weltstadtfauna (im Gegensatz zur Großstadtfauna) kann heute wohl (noch) nicht gesprochen werden. Bryobia kommt auch in kleinen und neuen Siedlungen vor.

DIE INSEKTENFAUNA DES WIENER STADTGEBIETES ALS BEISPIEL EINER KONTINENTALEN GROSS-STADTFAUNA

HARALD SCHWEIGER, Wien

Die Großstädte der Erde bilden wohl jenen Lebensraum, der den häufigsten und stärksten Störungen anthropogener Natur unterworfen wird. Obwohl alle diese Störungen irgendwie mit der Tätigkeit des Menschen zusammenhängen, wechseln sie in bezug auf Art, Häufigkeit und Stärke von Fall zu Fall derartig sprunghaft, daß es unmöglich erscheint, die Großstadt als einheitlichen Lebensraum aufzufassen. Selbst bei der Untersuchung kleinster Flächen in der geschlossen verbauten Zone (z. B. Reste von Hausgärten, aber auch Gebäude, Ruderalflächen usw.) begegnet einem öfters ein heterogenes Mosaik von verschiedenartigen Lebensräumen, deren ökologische Beurteilung und Einordnung manchmal recht schwer fällt (vgl. Kühnelt 1955, p. 30).

Die Verschiedenartigkeit der Lebensräume und der manchmal recht schnelle Wechsel der Umweltsfaktoren bedingt, daß die Faunen der verschiedenen Großstädte, auch wenn diese in der gleichen klimatischen Zone liegen, in artenmäßiger Hinsicht durchaus nicht gleichmäßig zusammengesetzt sind, sondern daß in dieser Beziehung öfters recht bedeutungsvolle Unterschiede bestehen können. Die Faunen der großen Städte stimmen lediglich in einem Punkt vollkommen überein, nämlich, daß hier nur solche Tierarten zu leben vermögen, die sich an die herrschenden extremen Verhältnisse vollkommen anpassen. Leider ist aber die Erforschung der Großstadtfauna (Großstadtbiologie) noch ein ganz junger Spezialzweig der biologischen Wissenschaften, und es existieren daher nur aus ganz wenigen Städten (z. B. Hamburg) erschöpfende Faunenlisten, welche sichere Vergleichsmöglichkeiten bieten. Es wird daher erst zu einem späteren Zeitpunkt möglich sein, die Faunen der einzelnen Großstädte miteinander zu vergleichen, um auf diese Weise die Gesetzmäßigkeiten der Entstehung und Genese der Großstadtfaunen in allgemeingültiger Weise abzuleiten. Aufgabe des vorliegenden Aufsatzes ist es daher, and Hand des vorhandenen Materiales die wesentlichen Züge der Wiener Großstadtfauna aufzuzeigen, die schon aus rein geographischen Gründen (Hamburg: Hafenstadt im atlantisch-maritimen Klimabereich, Wien: kontinentale Stadt an bedeutungsvoller Faunen- und Klimagrenze) von der Hamburger Fauna sehr stark abweicht.

Bei einer Gesamtbeurteilung der Fauna des Wiener Stadtgebietes muß man sich vor allem vor Augen halten, daß Wien hart an der Grenze von zwei völlig verschiedenartigen Faunengebieten liegt: der trockenwarmen Steppe (Pannonische Zone, nach Schweiger, 1953 und 1961) und dem mehr feuchtkühlen Bergland des Wienerwaldes, das in faunistischer Hinsicht bereits als Ausläufer des Voralpengebietes betrachtet werden muß. Diese Faunengrenze, die sich durch ganz Niederösterreich weiterverfolgen läßt, führt nun auf Wiener Boden mitten durch die westlichsten Außenbezirke, so daß zwar der größte Teil des Stadtgebietes noch im Einflußbereich der Pannonischen Zone liegt, der äußerste Westen aber bereits zum montan beeinflußten Wienerwaldgebiet gehört. Wie bedeutungsvoll die Unterschiede zwischen Steppen- und Wienerwaldfauna sind, zeigen die folgenden zwei Faunenlisten, welche aus je einem charakteristischen Biotop der östlichen und westlichen Randzone des Wiener Stadtgebietes stammen:

1. Vogelschutzgebiet in Neuwaldegg (westliche Randzone) mit typischer Buchenwaldvegetation, die ihren ursprünglichen Charakter in weitgehendem Maße bewahrt hat. Es wurde hier in einer feuchten Schlucht am 12. 6. 1952 moderndes Laub gesiebt und unter Steinen am Rande eines kleinen Waldbaches gesammelt. Außerdem wurden auch mehrere modernde Buchenstücke untersucht.

a) Montan: *Carabus irregularis* Fabr., *Trechus cardioderus pilisensis* Csiki, *Pterostichus metallicus* F., *Abax ovalis* Duft., *ater germanus* Schaub., *Molops austriacus* Ganglb., *Dreposciodes alpestris* Jeann., *Domene scabricollis* Er., *Laena viennensis* Sturm, *Rosalia alpina* L., *Otiorrhynchus bisulcatus* Fabr., *Liparus germanus* L., *Hypera palumbaria* Germ.

b) Weit verbreitet: *Carabus coriaceus* L., *Trechus quadristriatus* Schrank, *Agonum ruficorne* Goeze, *Pterostichus niger* Schall., *anthracinus* Illig., *Tachinus humeralis* Gravh., *Quedius picipennis* Payk. *Neuraphes elongatulus* Müll. et Kunze, *Dasycerus sulcatus* Brongn., *Enicmus minutus* L., *Cantharis fusca* L., *Phyllotreta tetrastigma* Com.

c) Südliche + südöstlich: *Leptura erratica* Dalm., *Stenopterus rufus* L.

2. Steppenartiger Grasplatz am Wienerberg (östliche Randzone) mit typisch pannonischer Vegetation. In der Umgebung zahlreiche Bauten vom Hamster (*Cricetus cricetus* L.). Am 12. 5. 1950 fand ich hier unter Steinen und auf Pflanzen folgende Koleopteren:

a) Pontisch + südöstlich: *Carabus hungaricus* F., *Harpalus sabulicola ponticus* Schaub., *Zabrus spinipes* Fabr., *Pterostichus marginalis* Dej., *Microlestes plagiatus* Duft., *Oxytelus intricatus* Er., *Philonthus spermophili* Ganglb., *Blaps halophila* Fisch., *Dorcadion aethiops* Scop., *fulvum* Scop., *pedestre* Poda, *Otiorrhynchus velutinus* Germ., *Psalidium maxillosum* F., *Omophlus proteus* Kirsch.

b) Mediterran + pontomediterran: *Harpalus cribricollis* Dej., *zabroides* Dej., *tenebrosus centralis* Schaub., *Acupalpus luteatus* Duft., *Metabletus pallipes* Dej., *Drypta dentata* Rossi, *Astrapaeus ulmi* Rossi, *Ctenistes palpalis* Reichenb., *Hister quadrimaculatus* L., *Formicomus pedestris* Rossi.

c) Weit verbreitet (xerophil bzw. beschränkt thermophil): *Calathus fuscipes* Goeze, *Pterostichus punctulatus* Schall., *cupreus* L., *Amara aenea* De Geer, *eurynota* Panz., *Harpalus pubescens* Müll., *affinis* Schrank, *distinguendus* Duft., *rubripes* Duft., *hirtipes* Panz., *Brachynus explodens* Duft., *crepitans* L., *Opatrum sabulosum* L., *Crypticus quisquilius* L., *Cleonus piger* Scop., *Mecaspis alternans* Hrbst.

Naturgemäß treten die Unterschiede zwischen der Steppen- und der mehr montan beeinflußten Waldfauna in den relativ naturbelassenen Biotopen der Randzone am deutlichsten zutage, während sie sich in den geschlossen versiedelten Zonen (Gartenland, vollständig verbautes Gebiet) mehr oder weniger verwischen. Aber auch innerhalb der Gartenfauna bestehen noch recht beachtliche Unterschiede, wenn man in faunistischer Hinsicht einen innerhalb der pannonischen Zone gelegenen Garten mit einem des westlichen Stadtgebietes vergleicht. Innerhalb der am stärksten anthropogen beeinflußten Biotope spielen im Wiener Stadtgebiet neben weitverbreiteten Formen allerdings nur die Bewohner der pannonischen Zone eine größere Rolle, da sich diese auch im geschlossen verbauten Gebiet an geeigneten Stellen in Massen ansiedeln

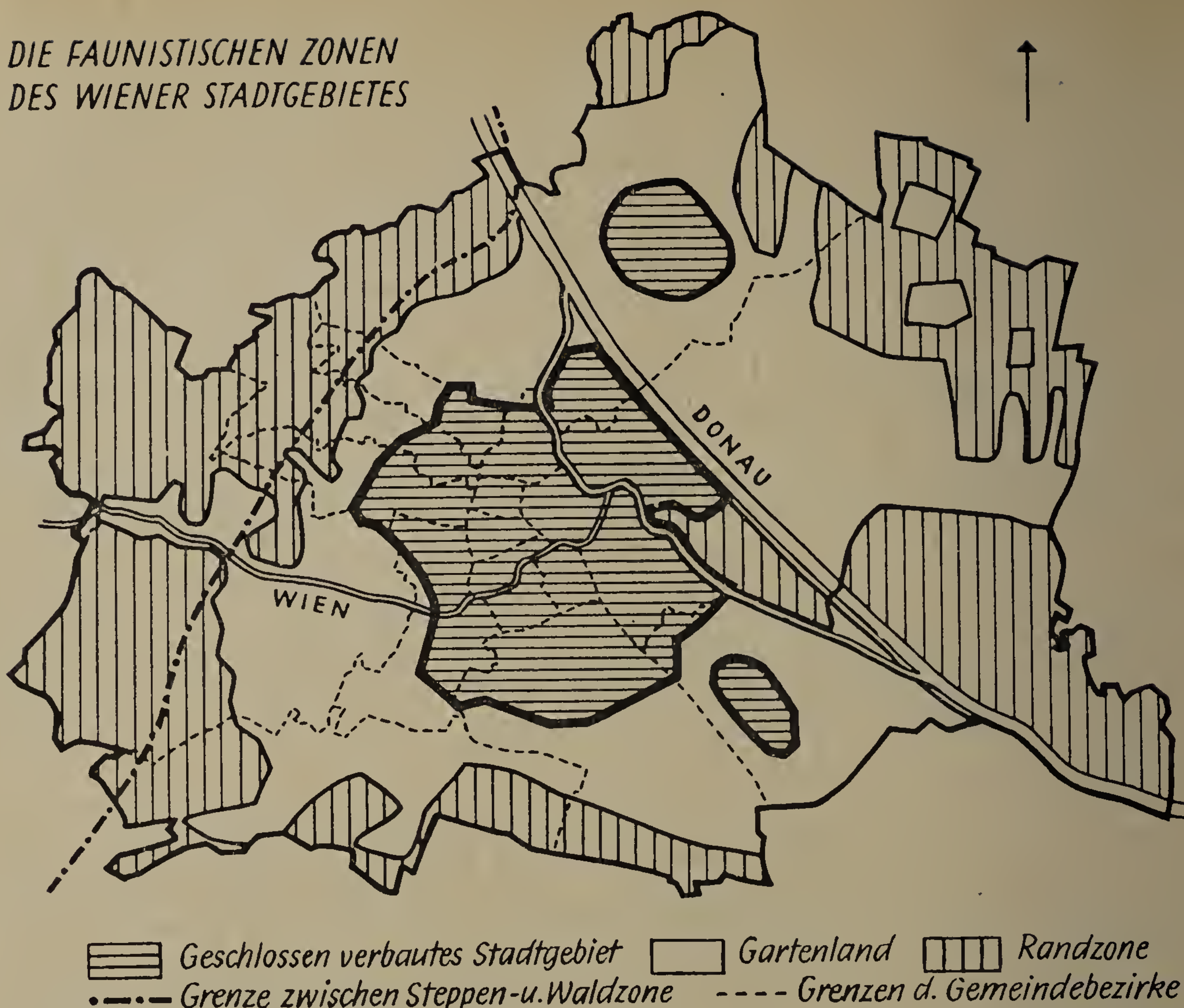

Abb. 1

können. So fand ich z. B. auf der trockenen Schutthalde einer im Stadtzentrum gelegenen Bombenruine in großer Anzahl die Laufkäfer *Harpalus zabroides* Dej., *puncticollis* Payk., ferner die Tenebrioniden *Opatrum sabulosum* L. und *Crypticus quisquilius* L. sowie verschiedene phytophage Arten, deren Vorkommen an *Artemisia campestris* gebunden ist (*Baris artemisiae* Hrbst., *Cuculia artemisiae* Hfng. usw.[1]). Die genannten Arten sind typische Bewohner von trockenen Grasplätzen, Feldrainen usw. Im Gegensatz zu den Angehörigen der xerophilen Artgruppe spielen meso- bzw. hygrophile Elemente (z. B. Uferkäfer) auf den Ruderalplätzen des verbauten Wiener Stadtgebietes nur eine sehr geringe Rolle, wodurch das Dominieren der Steppenbewohner noch deutlicher zutage tritt. Lediglich in manchen alten Parkanlagen (Augarten usw.) findet man noch vereinzelt Überreste einer ursprünglichen Waldfauna (*Carabus coriaceus* L., *germari exasperatus* Duft. usw.). An solchen Stellen können manchmal die xerophilen Elemente vollständig fehlen bzw. werden sie hier durch eine mesophile Fauna ersetzt. Das gleiche gilt auch für die alten Hausgärten im Stadtbereich.

Die Verarmung der Fauna

Wenn man von der heutigen politischen Grenze der Stadt Wien ausgehend gegen den vollständig verbauten Stadtkern zu wandert, kann man eine allmähliche Verarmung der Fauna feststellen. Innerhalb des Wiener Stadtgebietes lassen sich dabei drei große Zonen unterscheiden (Abb. 1):

1. Randzone,
2. Gartenland,
3. geschlossen verbautes Gebiet.

[1] Die ebenfalls an Artemisia gebundene Noctuide *Cuculia fraudatrix* Ev. wurde von mir in Floridsdorf bereits im Jahre 1953, also lange bevor noch weitere Fundorte aus Niederösterreich bekannt wurden, auf einer Ruderalstelle gesammelt (Belege in coll. nö. Landesmuseum).

VERARMUNG DER TIERWELT

GRUPPE	ANZAHL DER ARTEN		
	RANDZONE	GARTENLAND	VERBAUTES GEBIET
			
Carabus	17	7	2?
Trechus	6	3	1
Pterostichus	22	10	2
Euconnus	11	3	2
Dermestidae	24	15	27
Bombus	13	7	1
Acrididae	32	6	1
Tettigoniidae	20	4	1

Abb. 2

Die genannten drei Zonen unterscheiden sich voneinander hauptsächlich durch negative Faktoren: die Randzone besitzt die reichste, der Stadtkern die ärmste Fauna; während das Gartenland eine Mittelstellung einnimmt. Obwohl es sich auch bei der ringförmig um den Stadtkern gelagerten Gartenzone um einen extrem kulturbeeinflußten Lebensraum handelt, finden wir hier eine überraschend arten- und formenreiche Fauna, wobei jedoch eine einseitige Bevorzugung gewisser Tiergruppen unverkennbar ist.

Im Rahmen einer faunistischen Untersuchung in Floridsdorfer Hausgärten konnte ich nicht weniger als 350 verschiedene Käferarten nachweisen. Unter diesem Material fällt das Dominieren von phyto- und saprophagen Formen auf, während carnivore Arten deutlich zurücktreten. Xylophage Formen treten nur vereinzelt auf (z. B. Ipiden und Anobien). Ähnlich einseitig ist auch die Vogelfauna dieser Gärten zusammengesetzt.

Die schrittweise Verarmung der Fauna zeigen die verschiedensten Insektenordnungen in sehr anschaulicher Weise (Abb. 2). Während z. B. in der Randzone etwa 13 verschiedene Arten der Gattung *Bombus* L. vorkommen, finden wir im Gartenland nur mehr 7. In den großen Parkanlagen der Innenstadt lebt schließlich nur mehr eine einzige Art, nämlich *Bombus terrestris* L. Noch stärker treten diese Unterschiede in der artenmäßigen Verteilung der Gattung *Carabus* L. und bei den *Acridiidae* zutage.

Diese Verarmung der Fauna wird aber leicht verständlich, wenn man bedenkt, daß bereits das Gartenland als stark kulturbeeinflußte Zone für viele Bewohner der freien Landschaft (z. B. alle typischen Kulturflüchter) absolut unbewohnbar ist. Die geschlossen verbauten Zonen bilden schließlich einen extremen Lebensraum, der nur von ganz wenigen anpassungsfähigen Arten besiedelt werden kann.

Die Randzone

Als Randzone wird jenes Gebiet bezeichnet, das direkt an den ringförmigen Garten- und Siedlungsgürtel am Stadtrand anschließt und streng genommen nur mehr im geographischen Sinne zum Wiener Stadtgebiet gerechnet werden kann. In dieser Randzone findet man ausgedehnte Gebiete, die ihren ursprünglichen Charakter weit-

gehend bewahrt haben (Lobau, Lainzer Tiergarten, Abhänge des Leopoldsberges usw.), es wechseln hier Wiesen und Felder mit Steppenplätzen und gartenmäßig genutztem Kulturland, kurz, die Landschaft mit ihrer Vielzahl von Lebensräumen unterscheidet sich ebensowenig von ihrer bereits in Niederösterreich liegenden Umgebung, wie die hier vorkommende artenreiche Tier- und Pflanzenwelt. In der Randzone leben daher sehr viele Arten, die in den beiden übrigen Zonen fehlen.

Eine erschöpfende Behandlung der Randzonenfauna erübrigt sich, da diese Zone nicht zum engeren Stadtgebiet gehört. Es wird jedoch darauf verwiesen, daß es in der westlichen Randzone des Wiener Stadtgebietes einzelne Vorkommen von typisch montanen Arten gibt, die als äußerste Ausläufer der Alpen- und Voralpenfauna bis hierher reichen. Solche montane Arten, sind u. a. die Käfer: *Carabus irregularis* Fbr., *Cychrus attenuatus* Fabr., *Trechus cardioderus pilisensis* Csiki, *Pterostichus metallicus* Fabr., *Abax ater germanus* Schaubg., *ovalis* Duft., *Domene scabricollis* Er., *Rosalia alpina* L. usw. Auf die Faunengrenze, welche durch das westlichste Stadtgebiet verläuft, wurde bereits an früherer Stelle verwiesen.

Das Gartenland

Das Gartenland umgibt den geschlossen verbauten Stadtkern gleich einem grünen Ring und ist gegenüber der Randzone morphologisch nur sehr unscharf abgegrenzt, so daß man in dieser Beziehung alle möglichen Übergänge feststellen kann. Um so schärfer ausgeprägt ist dagegen die Grenze gegenüber der geschlossen verbauten Zone.

Die Fauna des Gartenlandes unterscheidet sich von der Randzonenfauna einmal dadurch, daß hier infolge der Nähe des Menschen und der räumlichen Beengtheit nur solche Tierarten zu leben vermögen, die sich weitgehendst anzupassen vermögen oder eine relativ geringe Körpergröße besitzen. Dementsprechend fehlen im Gartenland alle größeren Säugetiere und Vögel. Bei den Insekten treten dagegen diese Unterschiede nicht so auffällig zutage.

Aber es gibt noch einen anderen charakteristischen Punkt, durch den sich die Gartenfauna von der Randzonenfauna unterscheidet. Während in der Randzone jeder Biotop seine speziellen Arten besitzt, fehlen solche biotopeigene Arten im Gartenland fast vollständig. Mit anderen Worten: Im Gartenland lebt eine bunt gemischte Gesellschaft von Arten, die aus allen möglichen Lebensräumen der Randzone stammen.

Im Rahmen von faunistischen Untersuchungen in Floridsdorfer Gärten fand ich u. a. 40 verschiedene Arten von Carabiden. Von diesen 40 Arten leben 10 normalerweise in Wäldern (z. B. *Carabus germari pseudoviolaceus* Breun, *Pterostichus niger* Schall., *Synuchus nivalis* Panz., *Platynus assimilis* Payk.), 8 sind typische Uferbewohner (*Asaphidion flavipes* L., *Bembidion properans* Steph., *inoptatum* Schaum, *Agonum mülleri* Hbst. usw.), 11 Bewohner von trockenen Grasplätzen, Feldrainen und Ruderalstellen (*Calathus ambiguus* Payk., *Pterostichus cupreus* L., *Amara aenea* De Geer, *fulva* Dej., *Harpalus brevicollis* Serv., *azureus* Fabr., *pubescens* Müll., *atratus* Latr., *anxius* Duft., *Callistus lunatus* Fabr. usw.), während sich der Rest aus euryöken Arten zusammensetzt, die auch in der Randzone alle möglichen Lebensräume bewohnen (*Trechus quadristriatus* Schrank, *Pterostichus vulgaris* L., *Dolichus halensis* Schall. u. a.). Was nun die Verteilung der genannten Arten in den Gärten selbst betrifft, so konnte man zwar feststellen, daß die Bewohner trockener Biotope im allgemeinen solche Stellen auch in den Gärten aufsuchten, während die Waldformen mehr in schattigen Lagen (Hecken, mit Brennesseln und Holunder bestandenes Unland) auftraten. Trotzdem kam es aber nicht selten vor, daß unter ein und demselben Stein zu gleicher Zeit eine mehr xerophile Art mit einer typisch meso- bzw. hygrophilen Wald- oder Uferform beisammen saß (z. B. *Harpalus brevicollis* Serv. mit *Asaphidion flavipes* L. und *Synuchus nivalis* Panz. oder *Carabus germari pseudoviolaceus* Breun., *Calathus ambiguus* Payk. und *Bembidion inoptatum* Schaum), eine Erscheinung, die man in antochthonen Biotopen kaum beobachten kann.

Unter den Evertebraten des Gartenlandes spielen aber auch solche Arten eine Rolle, die durch den Menschen aus fremden Faunengebieten importiert wurden und die in der Folge festen Fuß fassen konnten (Adventivarten). Charakteristische Adventiv-

arten, die in den Wiener Gärten eine weite Verbreitung besitzen, sind u. a.: *Quadraspidiotus perniciosus* Comst., *Ceratitis capitata*, *Hiphantria cunea* Drury, *Perigona nigriceps* Dej., *Lithocharis nigriceps* Kraatz, *Lathridius nodifer* Westw., *Acanthoscelides obtectus* Say, *Leptinotarsa decemlineata* Say. Wie man sieht, befindet sich unter den Adventivarten eine nicht unerhebliche Anzahl von Schädlingen. Interessehalber sei noch darauf verwiesen, daß in den Wiener Gärten fallweise auch ein kleiner Blindkäfer (*Anommatus pannonicus* Kaszab) in größerer Anzahl vorkommt. Der Käfer lebt besonders in den Wurzelscheiben von Tulpen- und Lilienzwiebeln.

Das geschlossen verbaute Gebiet

Das geschlossen verbaute Gebiet (Stadtkern) bildet in ökologischer Hinsicht die extremste Zone. Hier reiht sich Häuserzeile an Häuserzeile, und der Asphalt der Straßen und Gehsteige ersetzt den Rasen der Gärten. Grünflächen sind auf ein Mindestmaß reduziert. Kleine Parks, einzelne Bäume, Reste von alten Hausgärten und mit einer oftmals üppigen Ruderalflora bewachsene Schuttplätze spielen die gleiche Rolle wie die Oasen in der Wüste. Trotzdem findet man manchmal auf engstem Raum zusammengedrängt ein relativ artenreiches Insektenleben, das sich aber nur aus solchen Arten zusammensetzt, welche die zwischen den einzelnen Grünflächen eingeschalteten breiten Barrieren aus Beton und Asphalt leicht überwinden können.

Alleen und Bahndämme bilden wichtige Einwanderungsstraßen, entlang derer die Insekten tief in die geschlossen verbaute Zone eindringen. So kann man z. B. die grüne Laubheuschrecke (*Locusta viridissima* L.) sogar noch in den Kronen der Ringstraßenbäume finden. Auf der Böschung des Nordwestbahndammes fand ich inmitten der geschlossen verbauten Zone des XX. Bezirkes u. a. *Bombus lapidarius* L., 3 Arten von Feldheuschrecken (*Acridiidae*), *Chrysomela analis* L. und *Geleruca tanaceti* L., alles Arten, die ansonsten im geschlossen verbauten Gebiet vollständig fehlen und die sicherlich nur unter Benützung des Bahndammes so tief gegen den Stadtkern vordringen konnten. Daß flugfähige und leicht bewegliche Formen hiebei besonders begünstigt werden, versteht sich von selbst.

Ein sehr formenreiches Insektenleben begegnet einem manchmal auch auf Schuttplätzen, wenn diese eine entsprechende Vegetation besitzen. So fand ich 1947 auf dem Schuttplatz einer Bombenruine (Wien II.!) innerhalb eines halben Jahres folgende Koleopteren:

Carabidae: *Notiophilus pusillus* Waterh., im feuchten Schutt unter Huflattich (Tussilago farfara); *Dyschirius globosus* Hbst., mit voriger Art zusammen; *Bembidion lampros* Hbst., *properans* Steph., *inoptatum* Schaum, alle im feuchten Schutt; *Asaphidion flavipes* L., unter Mörtel; *Trechus quadristriatus* Schrk., zwischen Graswurzeln; *Amara aenea* De Deer, *familiaris* Dft., *aulica* Panz. mit voriger Art zusammen; *Harpalus affinis* Schrank, *distinguendus* Dutt. unter Steinen; *Acupalpus meridianus* L. zwischen feinem Schutt; *Microlestes minutulus* Goeze, mit vorigem zusammen; *Idiochroma dorsalis* Pont., zwischen einzelnen Grasbüscheln.

Staphylinidae: *Falagria sulcata* Payk., unter faulenden Huflattichblättern; *Astilbus canaliculatus* F., mit *Idiochroma dorsalis* Pont. zusammen; *Oligota pusillima* Grav., im Sande zwischen Schutt; *Philonthus politus* L., zwischen Graswurzeln; *Gabrius vernalis* Grav., mit vorigem zusammen; *Medon obscurellus* Er., unter Huflattich; *Lithocharis nigriceps* Kr., mit vorigem zusammen; *Stilicus rufipes* Germ., unter Schutt; *Stenus ater* Mannh., an feuchteren Stellen unter Schutt; *circularis* Grav., mit vorigem zusammen; *Bledius fracticornis* Payk., zwischen Huflattichpflanzen; *Oxytelus insecatus* Grav., *nitidulus* Grav. und *tetracarinatus* Block, unter feuchtem Schutt; *Trogophloeus elongatulus* Er., mit vorigen zusammen.

Silphidae: *Ptomophagus sericatus* Rosenh., unter faulendem Detritus.

Ptiliidae: *Ptenidium pusillum* Gyll., mit vorigem zusammen; *Acrotrichis grandicollis* Mnnh., aus Graswurzeln; *intermedia* Gillm., ebenda.

Histeridae: *Hister quadrinotatus* Scr., unter Steinen (1); *unicolor* L., ebenda; *carbonarius* Illig., zwischen faulenden Huflattichblättern; *Onthophilus striatus* Forst., ebenda.

Scarabaeidae: *Trox scaber* L., in trockenem Detritus; *Pleurophorus caesus* Panz., in feuchtem Schutt; *Oxyomus silvestris* Scop., ebenda; *Aphodius haemorrhoidalis* L., zwischen Gras; *prodromus* Brahm, auf frischem Hundekot; *Onthophagus caenobita* Hrbst., ebenda (1); *Amphimallon solstitialis* L., am Abend herumschwärmend; *Epicometis hirta* Poda, auf Leontodonblüten (2).

Hydrophilidae: *Helophorus nubilis* F., in feuchter Erde; *Cercyon unipunctatus* L., ebenda.

Byturidae: *Byturus aestivus* L., auf Leontodonblüten.

Nitidulidae: *Meligethes aeneus* Fabr., auf Leontodonblüten;

Cryptophagidae: *Cryptophagus cellaris* Scop., unter Detritus (1) *Atomaria linearis* Steph., ebenda; *atricapilla* Steph., ebenda.

Lathridiidae: *Enicmus minutus* L., *transversus* Oliv., *Cortycaria pubescens* Gyllh., *Corticarina gibbosa* Hrbst., alle in mehr oder weniger feuchtem Detritus und an schimmeligem Holz.

Byrrhidae: *Cytilus sericeus* Forster, zwischen Graswurzeln.

Malachiidae: *Axinotarsus pulicarius* Fbr., von Lamium gekötschert; *Malachius aeneus* L., auf Leontodonblüten; *Dasytes plumbeus* Müll., ebenda.

Corynetidae: *Corynetes meridionalis* Obbg., auf einer Mauer (1); *coeruleus* De Geer, auf Lamium (2).

Anthicidae: *Notoxus monoceros* L., auf Lamium; *Formicomus pedestris* Rossi, unter trockenem Schutt; *Anthicus bifasciatus* Rossi, ebenda: *quisquilius* Thoms., ebenda.

Chrysomelidae: *Melasoma populi* L., auf Populus alba; *Chalcoides aurata* Marsh., ebenda; *Epithrix pubescens* Koch, auf Solanum nigrum L.; *Chaetocnema aridella* Payk., auf Gras; *Phyllotreta undulata* Kutsch., *nigripes* F., beide auf Sinapis arvensis; *Longitarsus gracilis* Kutsch., im September auf Tussilago farfara in Massen; *Sphaeroderma testaceum* Fabr. auf Disteln; *Psylliodes chrysocephala* L., auf Sinapis; *affinis* Payk., auf Solanum nigrum; *chalcomera* Illig., auf Carduus. Alle *Halticinae* det. Rag. Rat. F. Heikertinger, Wien †.

Curculionidae: *Phyllobius arborator* Hrbst., auf Populus alba; *piri* L., ebenda; *argentatus* L., ebenda und an Acer negundo; *Sitona humeralis* Steph., auf Gras; *Ceutorrhynchus litura* Fabr., auf Carduus; *Apion aeneum* Fabr., auf Malva. Alle det. L. Magnano, Verona.

Sobald die Ruderalflächen eine größere Ausdehnung besitzen, werden sie nicht selten von Pflanzengesellschaften besiedelt, die hinsichtlich ihrer artlichen Zusammensetzung sehr stark an die pannonischen Feldfluren und Grasplätze erinnern. Entsprechend der Flora lebt an solchen Stellen auch eine Insektenfauna mit betont xerophilem Einschlag. So fand ich auf einer größeren, im Wiener Stadtzentrum schon seit Jahrzehnten bestehenden Ruderalfläche u. a. die Käfer: *Harpalus zabroides* Dej., *puncticollis* Payk., *calceatus* Duft., *Opatrum sabulosum* L., *Crypticus quisquilius* L. und *Baris artemisiae* Hrbst. in Anzahl, alles Arten, die ansonsten xerotherme Standorte bevorzugen.

Ein ganz anderes Bild bieten in faunistischer Hinsicht die großen Parkanlagen im Herzen von Wien mit ihren alten Baumbeständen. Hier finden sich besonders unter den xylophagen Formen manchmal auch richtige Wald- und Auwaldrelikte. So fing ich im Wiener Volksgarten die Käfer *Prionychus ater* Fabr. und *Aegosoma scabricorne* Scop., beides Arten, die an alte Bäume gebunden sind. Ähnlich erklärt sich auch das Vorkommen von *Cetonia aurata* L. und *Potosia aeruginosa* Drury, die beide manchmal im Rathauspark bzw. Stadtpark gefunden werden. Im Gegensatz zu den an alte Bäume gebundenen Formen muß die Fauna der großen Rasenflächen als einförmig und artenarm angesprochen werden. Unter den Käfern dominieren hier die Carabiden *Amara aenea* De Geer und *familiaris* Duft. Außerdem finden sich noch verschiedene kleine *Apion*-Arten sowie *Longitarusu pratensis* Panz. und *melanocephalus* Deg., die an bestimmte, hier häufig vorkommende Pflanzenarten (Trifolium, Plantago) gebunden sind. Erwähnt sei noch das Vorkommen von *Bombus terrestris* L. und *Chorthippus biguttulus* L., da in der geschlossen verbauten Zone ansonsten keine weiteren *Bombus* bzw. *Acrididae* vorkommen. Bei den sich auf den Rasenflächen manchmal in größerer Anzahl tummelnden Tagfaltern (z. B. *Pieris brassicae* L., *rapae* L., *Colias hyale* L., *croceus* Fourc., *Vanessa cardui* L., *Aglais urticae* L.) handelt es sich fast ausschließlich um von auswärts zugeflogene Exemplare.

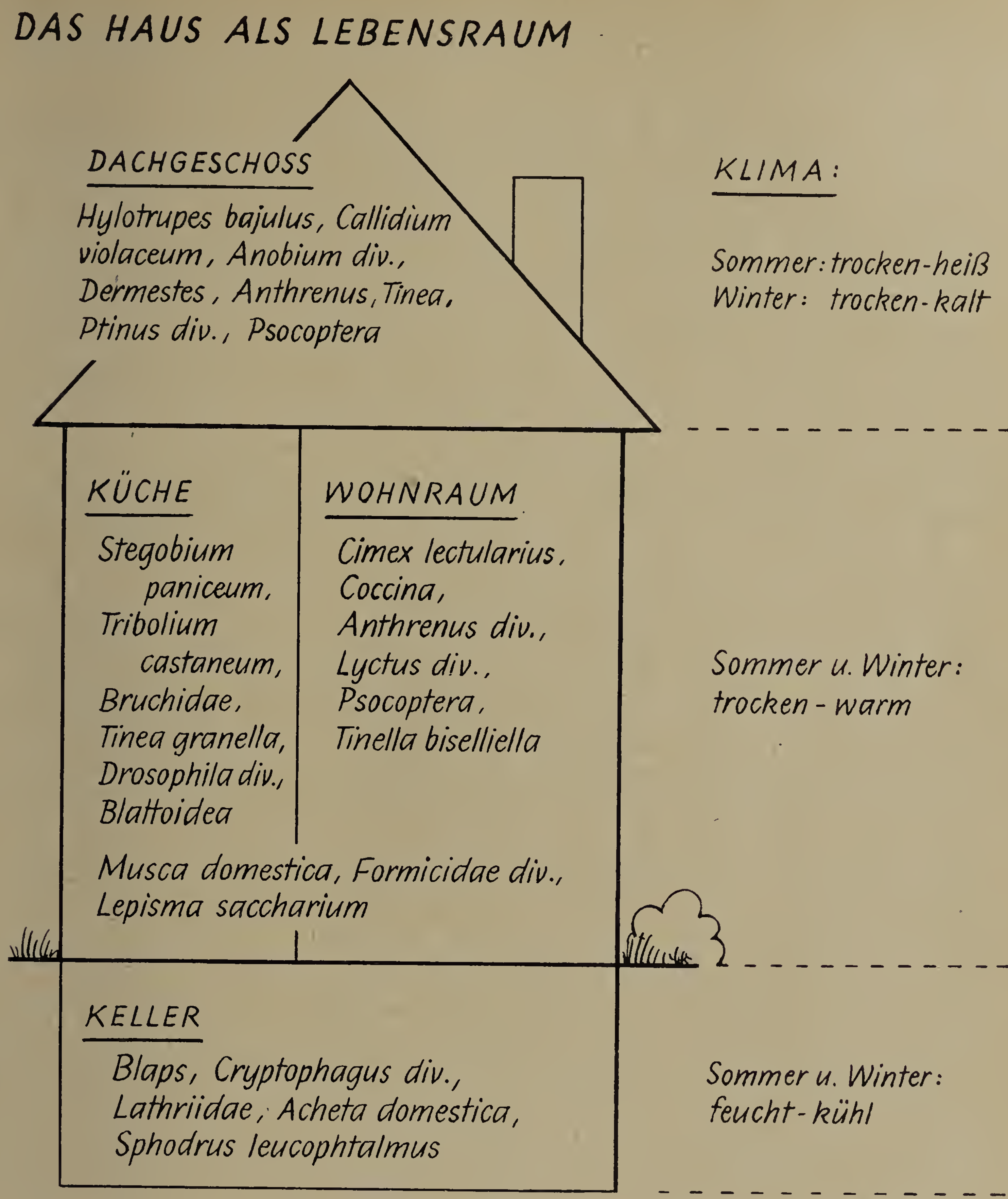

Abb. 3

Den wichtigsten Lebensraum der Insekten bilden aber in der geschlossen verbauten Zone die vom Menschen errichteten Gebäude. Doch auch diese bilden in ökologischer Hinsicht keine Einheit, sondern setzen sich aus einer Vielzahl von kleinen und kleinsten Biotopen mit sehr heterogenen Lebensbedingungen zusammen. Von unten nach oben steigend kann man z. B. in Wohnhäusern drei Gruppen von Räumen unterscheiden, die alle eine einigermaßen charakteristische Tierwelt besitzen (Abb. 3):

a) Keller,
b) Wohnräume,
c) Dachboden.

So finden wir in feuchten Kellern eine Fauna, die viele Elemente enthält, welche auch in Höhlen bzw. Kleinsäugerbauten vorkommen, wie z. B. *Trechus austriacus* Dej.,

der in verschiedenen niederösterreichischen Höhlen zu den häufigsten Erscheinungen zählt oder *Blaps lethifera* Marsh., der in Massen in Kaninchenbauten lebt. Ein ganz anderes Bild bieten die trockenen Keller der zentralgeheizten Neubauten, in denen eine äußerst artenarme Fauna (z. B. *Thermobia domestica* Pack.) lebt.

Im Gegensatz zum Keller besitzen die Wohnräume ein warmes ausgeglichenes Klima und je nach ihrer Verwendung eine recht unterschiedliche Fauna. So dominieren in der Küche in artenmäßiger Hinsicht diverse Vorratsschädlinge und Arten, die von Speiseresten und Abfällen leben (*Formicidae*, *Gryllulus domesticus* L., div. *Bruchiden* usw.). Im Wohn- und Schlafzimmer fehlen normalerweise solche Nahrungsquellen, weshalb hier die meisten typischen Küchenbewohner auf die Dauer nicht existieren können. An ihre Stelle treten vielmehr neben Ungeziefer (*Cimex lectularius* L., *Ctenocephalides canis* Curt. und *felis* Bouche) vor allem Trockenholzzerstörer (*Anobium*, *Lyctus*) und Textilienschädlinge (*Tineola biselliella* Humm.). Eine wichtige Rolle spielen auch Arten, die von feinstem organischem Detritus leben (z. B. *Lepisma saccharinum* L., *Psocoptera*) und gewisse spezialisierte Räuber (*Reduvius personatus* F.). Reich entwickelt ist auch die Dipterenfauna, wobei diverse *Drosophila*-Arten (*melanogaster* Meig., *obscura* Fall., *hyddei* Sturt. usw., alle det. Basden, Edinborough) und verschiedene Musciden (*Musca domestica* L., *corvina* F., *Fannia canicularis* L., *Sarcophaga* sp., *Lucilia caesar* L., *Calliphora* sp., vereinzelt auch *Stomoxys calcitrans* L.) zahlenmäßig dominieren.

Aber die Unterschiede gehen noch viel weiter. In einem feuchten muffigen Wohnzimmer leben ganz andere Arten (*Lathridiidae*, *Cryptophagus* usw.) als in einem trockenen (*Anthrenus olgae* Kalik, *museorum* L.). Ebenso ist es für die Zusammensetzung der „Wohnungsfauna" von allergrößter Bedeutung, ob die Hausfrau eifrig mit Scheuermittel und Bodenwachs hantiert, oder ob die Wohnung vor Schmutz starrt: Die reichste Fauna lebt nämlich in den schmutzigsten Wohnungen, denn Staub und Spinnweben bieten vielen Insekten hervorragende Lebensbedingungen (*Ptinus*, Flohlarven, *Lepisma* usw.).

Als letzter und in klimatischer Hinsicht extremster Lebensraum sei schließlich der Dachboden behandelt. Hier ist es im Sommer glühend heiß und im Winter bitter kalt. Entsprechend diesen extremen Verhältnissen ist auch die Fauna zusammengesetzt, die ein xerophiles Gepräge aufweist. Im Holze der Dachstühle dominieren *Anobium punctatum* De Geer und *Hylotrupes bajulus* L. als Trockenholzzerstörer. Seltener treten *Opilo domesticus* Sturm und *Anthocomus bipunctatus* Harrer auf, welche beide holzbewohnende Insekten und deren Larven räuberisch verfolgen. Von abgestorbenen Insekten leben auch die fallweise zu beobachtenden *Necrobia*- (*violacea* L. und seltener auch *rufipes* De Geer) und *Ptinus*-Arten (hier hauptsächlich *fur* L. und *pusillus* Sturm). In alten Kleidern und Lumpen siedeln sich schließlich nicht selten Motten an (*Tinea pellionella* L., *Tineola biselliella* Hum., seltener *Trichophaga tapetzella* L. und *Monopis rusticella* Hübn.).

Befinden sich in den Bodenräumen Spatzen- oder Taubennester, dann erfährt die Fauna eine zusätzliche Bereicherung. In den Nestern von Haustauben (*Columba livia f. domestica*) fand ich auf Wiener Dachböden u. a.: *Forficula auricularia* L., div. *Psocoptera*, *Cimex columbarius* Jenyns, *Dermestes frischi* Kugel., *lardarius* L., *Anthrenus pimpinellae* F., *Necrobia violacea* L., *Tenebrio molitor* L., *obscurus* Fabr., *Tinea pellionella* L., *Ceratophilus gallinae* Schr. und *Argas reflexus* F. Nicht unerwähnt möge schließlich die Tatsache bleiben, daß auch die Dachböden von verschiedenen Insekten als Überwinterungsort auserwählt werden (z. B. *Vanessa urticae* L., *Calliphora* sp.).

Infolge der günstigen klimatischen Bedingungen können sich im Bereich der menschlichen Behausungen auch Arten halten, deren eigentliche Heimat in viel wärmeren Gebieten liegt und die daher in unseren Breiten im Freiland unfehlbar zugrunde gehen müßten. Solche Arten wären z. B. *Thermobia domestica* Pack., *Acheta domestica* L., *Stegobium paniceum* L., *Ephestia kühniella* Zell. usw. Es muß aber festgestellt werden, daß im Wiener

Stadtgebiet die Gesamtzahl der aus fremden Faunengebieten eingeschleppten und dauernd bzw. fallweise eingebürgerten Insektenarten wesentlich geringer ist als in Hamburg, was hauptsächlich auf die in dieser Hinsicht ungünstigere Lage von Wien (Binnenstadt!) zurückzuführen sein dürfte.

Abschließend sei noch darauf verwiesen, daß auch auf den isolierten Grünflächen allerkleinsten Ausmaßes, wie man sie fallweise in alten Hinterhöfen antrifft, eine relativ spezifische Insektenfauna lebt. Hier findet man vor allem die Ameise *Tetramorium caespitum* L., die manchmal auch in Wohnungen eindringt, ferner regelmäßig die Käfer *Trechus quadristriatus* Schrank, *Xantholinus longiventris* Heer, *Tachyporus pusillus* Grav. und, wenn reichlich Moos vorhanden ist, auch *Cytilus sericeus* Forst. Auf den hier manchmal wachsenden Ailanthusbäumen macht der aus Ostasien stammende und im zentralen Wiener Stadtgebiet recht häufige Ailanthusspinner (*Philosamia cynthia* L.) seine gesamte Entwicklung durch (vgl. Schremmer 1954).

LITERATURVERZEICHNIS

Aus Gründen der Raumersparnis werden hier nur einige zusammenfassende Arbeiten angeführt, die durch ihren Inhalt ein rasches Einarbeiten in die behandelte Materie ermöglichen. Umfassende Literaturverzeichnisse finden sich u. a. bei Kühnelt 1955, Schremmer 1954, Schweiger 1953 und Weidner 1952.

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DISKUSSION

L. P. LEFKOVITCH: *Lasius brunneus* appears to be becoming more common in houses in southern England although still rare in this habitat.

K. W. HARDE: Auch für Stuttgart wird in den letzten Jahren eine starke Zunahme von *Niptus hololeucus* bestätigt.

STORED PRODUCTS ENTOMOLOGY IN PORTS

J. A. FREEMAN

Manuskript nicht eingelangt

ABSTRACT

Insects associated with stored products in ports are found in three main environments. These are the ships in which goods are carried to or from other countries: the transit sheds in which goods remain for short periods, and the warehouses and processing factories where goods may remain for long periods or may be converted into other products. The main features of each environment are described, with special reference to conditions in the United Kingdom and to the risks of introduction and establishment of new pests.

CONTROL OF TENT CATERPILLARS (Malacosoma neustrium L.) WITH A DUST MIXTURE OF BACILLUS THURINGIENSIS ON ELM TREES IN THE CITY OF AMSTERDAM

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The numerous canals of the City of Amsterdam are bordered with about 15,000 elm trees (*Ulmus* spp.). Periodically these trees suffer from damage by caterpillars by defoliation (Kalshoven, 1959). In the years around 1935 the brown tail moth (*Euproctis chrysorrhoea* Don.) was the main cause. In later years tent caterpillars (*Malacosoma neustrium* L.) were prevailing. Since 1938 the trees are dusted every springtime with a dust mixture of derris powder, containing 2% rotenone, which is rather effective against the young larvae. It takes one kilogram to treat three trees, sometimes two or three treatments are necessary and annually about 5,000 to 15,000 kg dust are used.

This practice exists up till to-day, notwithstanding numerous other and more effective insecticides are discovered, especially after the war. Although the treatments are given very early in the morning, even insecticides as DDT or malathion are considered too toxic to man, animals, foodstuffs and all things which are found along the streets of the city.

For some years attention has been paid to find an effective insecticide which is harmless to men and animals, and more effective to the Tent caterpillars as derris powder. Van Damme & Van der Laan (1959) tried a powder derived from the crystalliferous sporeforming bacteria *Bacillus thuringiensis* Berliner (Steinhaus, 1949; Lemoigne et al, 1956; Bucher, 1960). Larvae of *M. neustrium* proved in laboratory tests very susceptible to treatments with preparates from this bacteria. As is found by Heimpel & Angus (1959), a toxicant from the crystalls makes the mid-gut inactive and feeding is stopped within a few hours after treatment.

This insecticide has a great advantage to our scope: it is highly specific in his action, pathogenic only against some larvae of Lepidoptera, and harmless to other living organisms, including man, animals, plants, etc. (Steinhaus, 1957). Killing of the caterpillars occurs rather slow, but the larvae cease with feeding already a few hours after treatment, and no more damage is done.

Although the mode of action is not yet wholly cleared, it is highly probable that the bacteria are not acting as a biological agent, as no multiplication of the bacteria inside the caterpillars is found. The action may be compared with that of the antibiotics.

Springtime 1960 showed an uprise of the population of the tent caterpillars and many trees were defoliated, creeping caterpillars on the houses caused much trouble and windows gave trouble with opening because of the spinning pupating larvae.

A whole street with 150 elm trees of age of 15 years was treated twice with Bactospéine I. P. 54 (900,000 U. B./g), diluted with talc to 10% with a motorduster, 50 kg mixture per treatment being used. The effect seemed promising but heavy showers, occurring both times immediately after the treatments (10 and 14 June), spoiled much of the evidence, as the caterpillars were washed off earlier through the rain than through the toxicant. However, cage experiments corroborated once more very clearly: of 600 caterpillars treated, only 7% survived, whereas the untreated checks had a percentage of survivors of 82%..

Summarizing may be said that the prospects of control of this pest with products of *Bacillus thuringiensis* are favourable. The use of this specific insecticide should be seriously investigated in each case where the disadvantages of the commonly used, highly toxic all-over insecticides are appearing.

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DIE TRÜMMERFAUNA BRAUNSCHWEIGS

Ein Beitrag zur Frage der Auswirkung brachliegender Trümmergebiete auf die städtische Gemeinschaft des Menschen

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Die Tierwelt einer größeren Stadt pflegt sich im allgemeinen aus verschiedenen Elementen der in dem betreffenden Gebiet vorkommenden Fauna zusammenzusetzen, vermehrt durch mehr oder minder zahlreiche Arten, die entweder die Nähe des Menschen aktiv aufsuchen oder meist passiv infolge des in immer fernere Gebiete reichenden Handelsverkehrs bei ständig rascher werdenden Verkehrsverhältnissen in unsere Städte eingeschleppt werden und dort in unseren Breiten oft allein bestehen können, weil sie durch die beheizten Gebäude vor den Unbilden der kalten Jahreszeit geschützt sind. Der Mensch hat ihm schädliche oder lästige Tierarten in seinen Anwesen seit jeher zu vernichten versucht, sie zwar nicht immer ausrotten können, aber meist durch seine Bekämpfungsmaßnahmen erreicht, daß sie nicht überhandnehmen. So findet sich in der Stadt gewöhnlich eine charakteristische Tiergenossenschaft zusammen.

Eine zeitweise beträchtliche Veränderung in der Zusammensetzung der Fauna europäischer Städte wurde im zweiten Weltkrieg durch deren oft erhebliche Zerstörung und die Entstehung weiter Trümmerflächen hervorgerufen. Das war auch in der deutschen Stadt Braunschweig mit ihrem ausgedehnten Stadtkern aus alten Fachwerkhäusern der Fall, von der am 15. Oktober 1944 der größte Teil der Innenstadt sowie Teile der Vorstädte durch einen einmaligen Luftangriff weitgehend zerstört worden sind. Es entstand ein umfangreiches Trümmerareal, durch das ein neuer Lebensraum für die Tiere in der Stadt geschaffen wurde. Die Trümmerfauna Braunschweigs ist während annähernd 10 Jahren vom Zoologischen Institut in Braunschweig eingehend untersucht und unter Kontrolle gehalten worden.

Im allgemeinen ist die Trümmerfauna als arm zu bezeichnen, was hauptsächlich darauf zurückzuführen ist, daß die Trümmerzone den Tieren ursprünglich wenig zu fressen bietet. Erst wenn sich auf den Trümmerfeldern ein gewisser Pflanzenwuchs ausgebreitet hat und sich mit der Zeit spärliche Humusansammlungen bildeten, nahm die Tierwelt

an Artenzahl und meist auch an Individuen zu. Das gilt sowohl für die unmittelbar sich von pflanzlichen Stoffen nährenden Tiere als auch für die auf diese angewiesenen Räuber.

Spätestens in dem auf die Stadtzerstörung folgenden Winter mußten im Trümmergebiet sämtliche auf eine Überwinterung in beheizten Räumen angewiesenen Faunenelemente vollständig verschwinden. Erst als nach dem Krieg die Fernheizung wieder in Betrieb genommen worden war, drangen entlang der Heizungsrohre einige wärmebedürftige Arten in die Trümmerzone vor, zuerst das Heimchen, *Acheta domesticus* (L.) und die thermophile Assel *Metoponorthus pruinosus* (Brandt).

Schon durch das Fehlen vieler temperaturempfindlicher, eingeschleppter Arten überwog in der Trümmerfauna die Zahl der in dem Gebiet einheimischen Tiere. In der Innenstadt hatten oft Glieder der ursprünglichen Fauna des Landes bereits ein Refugium in Parks und größeren Gärten gefunden, falls sie die Betätigung des Menschen dort ertrugen. Das trifft vor allem bei Nachttieren zu, die zu der Zeit der Gartenarbeiten nicht aktiv sind, oder bei solchen Arten, die die infolge Kulturmaßnahmen in ihre Bestände gerissenen Lücken durch starke Vermehrung ausgleichen können. Sie haben mitunter sogar gewisse Vorteile eingetauscht, wie etwa günstigere Nahrungsverhältnisse oder Schutz vor manchen Feinden, ferner feuchtigkeitsliebende Tiere, wie Schnekken und Würmer, auch die Bewahrung vor großer Trockenheit, wie sie das regelmäßige Begießen der Pflanzenkulturen gewährleisten. Aber gerade der Mangel an ständiger Feuchtigkeit und an humösen Böden im Trümmergebiet mag die Ursache sein, daß Schnecken (*Gastropoda*), Regenwürmer (*Lumbricidae*) und Tausendfüßer (*Diplopoda*) die Trümmerzone weitgehend meiden, obwohl sie in den eingestreuten Gärten inmitten von Trümmern nicht selten vorkommen. Verschiedene bewegliche Tiere, wie vor allem Vögel, die in den städtischen Gärten infolge Schutz und reichlicher Nahrung oft eine hohe Siedlungsdichte und stärkere Bruten als außerhalb haben, besuchen aber vorübergehend die bewachsenen Teile der im späteren Stadium von Pflanzen bestandenen Trümmerzone zwecks Nahrungsaufnahme; dort fliegen gelegentlich auch Schmetterlinge und andere Insekten, die ihre Entwicklung offensichtlich in Parks und Gärten durchlaufen haben.

In höherem Maß für ein Eindringen in das Trümmergebiet waren jedoch wohl solche Tiere begünstigt, die in den Höfen und unter Steinen in der Stadt leben konnten. Erstaunlich rasch wurde die Trümmerzone Braunschweigs außerdem über die Vororte von außerhalb durch Elemente der dort vertretenen Fauna besiedelt, die wohl einst das Stadtgebiet vor dem Menschen hatten räumen müssen. Am meisten fielen darunter naturgemäß die auftretenden Säugetiere auf; sogar zwei Füchse, *Vulpes vulpes* (L.), wurden in der Trümmerzone Braunschweigs geschossen.

In den zerstörten Gebieten Braunschweigs entstanden sowohl Abschnitte mit homogenem Trümmerschutt von ausgesprochen sandig-erdigem Charakter, ohne nennenswerte steinige Bestandteile, als auch Anhäufungen von fast völlig erhaltenen Ziegelsteinen, wobei der zusammen mit ihnen aufgetretene Feinschutt im Verlauf der Zeit vielfach fortgespült worden war. Die Masse der Trümmer steht in ihrer Beschaffenheit zwischen den beiden Extremen. Im porösen Trümmerschutt versickern nun die Niederschläge rasch, so daß die oberste Bodenschicht, der Lebensraum der Pflanzenwurzeln und der Bodentiere, in kurzer Zeit austrocknet. Andererseits wird der kapillare Aufstieg des Grundwassers, in Gang gehalten durch die Verdunstung an der Bodenoberfläche, infolge der Großporigkeit des Substrats erschwert, oft sogar durch unterlagernde Fundamente oder Steinpflaster völlig unterbunden. Die Porosität der Trümmer ist aber in hohem Maß von dem Material abhängig, aus dem die Trümmer entstanden sind, sowie von ihrer Korngröße. In der Entstehung der Trümmer aus andersartigen Materialien werden wohl teilweise die unterschiedlichen Angaben für manche Städte

in der Trümmerbesiedlung mit feuchtigkeitsliebenden Tieren ihre Erklärung finden; während beispielsweise im Braunschweiger Trümmergebiet Landschnecken vollständig fehlen, wurde in demjenigen von Kiel das Vorkommen mehrerer Arten dieser Tiere festgestellt.

Die zunächst mehr oder weniger gleichmäßig verteilte Trümmermasse wurde in Braunschweig mit der Zeit an bestimmte Stellen massiert, so daß umfangreiche Schutthalden entstanden. Hier war eine Trümmerflora und -fauna oft besonders reichhaltig entwickelt. An solchen Halden siedelten sich auch mitten in der Stadt umfangreiche Populationen von Kaninchen, *Oryctolagus cuniculus* (L.), an, die nachts, wenn der Verkehr ruhte, nicht selten selbst größere Straßen der belebten Innenstadt kreuzten.

Eine bemerkenswerte ökologische Beobachtung ist die durch R. Finkbein im Braunschweiger Institut gemachte Feststellung, daß bestimmte Stellen des Trümmergebietes in Braunschweig erhebliche Salzanreicherungen aufwiesen, die vermutlich mit dem Verbrennen beträchtlicher Holzmengen bei Zerstörungen der mittelalterlichen Fachwerkbauten zusammenhängen. Schon nach kurzer Zeit sammelten sich an solchen Stellen salzliebende Pflanzen und Tiere an, Käfer und Wanzen, die auch an den Salzstellen in der Umgebung Braunschweigs vorkommen; häufig waren dort aber in der Stadt allein die Rübenwanze, *Piesma quadrata* (Fieber).

Da allgemein in den Trümmergebieten der Städte sich von Pflanzen ganz bestimmte Arten anzusiedeln vermögen und man daher eine spezielle „Trümmerflora“ unterscheiden kann, wirkte sich diese Zusammensetzung des Pflanzenbewuchses naturgemäß mitunter auch auf die Fauna aus. So konnten Tiere, vor allem Insekten, die an weitverbreitete Vertreter der Trümmerflora gebunden sind, durch deren Überhandnehmen leicht an Boden gewinnen und häufige Glieder der Trümmerfauna werden. Beispielsweise hat sich in den Trümmern mitteleuropäischer Städte das Weidenröschen, *Epilobium angustifolium* L., stark vermehrt; als Folge konnte der früher nicht in der Stadt zu findende und auch sonst im Gebiet nicht häufige Mittlere Weinschwärmer, *Choerocampa elpenor* (L.), in Braunschweig während der Nachkriegsjahre stellenweise häufig beobachtet werden. Auch der bereits früher in der Stadt Braunschweig regelmäßig vorkommende Ligusterschwärmer, *Sphinx ligustri* L., trat in den Trümmergebieten infolge der dort vorhandenen, wohl aus Gärten stammenden Exemplare seiner Wirtspflanze, die ungestört wachsen konnten, vermehrt auf.

Es ist hier nicht der Raum, die in annähernd 275 Arten in der Trümmerzone Braunschweigs im Laufe der Jahre angetroffenen Tiere, die in mehr als Einzelexemplaren festgestellt worden sind, in umfangreiche Listen zusammenzustellen oder gar einzeln zu besprechen, vor allem, da die Mehrzahl der Arten mehr oder minder Zufallsgäste sein dürften. Wichtiger scheint es, gewisse Leitformen zu nennen, die zahlreich gefunden wurden und die für das Trümmergebiet Braunschweigs als charakteristisch zu gelten haben. Es sind nicht allzu viele Species von Arthropoden und Vertebraten, die in diesem extremen Biotop offenbar optimale Lebensbedingungen gefunden haben; es handelt sich ausschließlich um euryöke Tiere mit weiter Verbreitung. Als allgemein häufig sind anzusprechen verschiedene Arten von Isopoda, hauptsächlich *Porcellio scaber* Latreille und *Oniscus asellus* L., von Chilopoda vor allem *Lithobius forficatus* (L.) und *Lithobius melanops* Newport, von Collembola *Hypogastrura armata* (Nicolet) und *Tullbergia krausbaueri* (C. Börner), von Coleoptera die Laufkäfer *Amara consularis* (Duftschmid), *Harpalus aeneus* (Fabricius) und *Acupalpus meridianus* (L.) sowie der Rapsglanzkäfer *Meligethes aeneus* (Fabricius), von Heteroptera vor allem *Orthotylus flavosparsus* (Sahlberg), *Campylomma verbasci* (Herrich-Schäffer) und *Nysius senecionis* (Schilling), von Lepidoptera die Klee-Eule, *Scotogramma trifolii* (Rottemburg), der Mittlere Weinschwärmer, *Choerocampa elpenor* (L.), und der Ligusterschwärmer, *Sphinx ligustri* L., von Diptera einige Arten der Fliegengattungen *Fannia* Robineau-Desvoidy,

Hydrotaea Robineau-Desvoidy und *Phaonia* Robineau-Desvoidy, ferner die Mücken *Petaurista maculipennis* (Meigen) und *Culex pipiens* L. Zu erwähnen ist, daß Spinnentiere in den Trümmergebieten Braunschweigs auffallend spärlich vertreten sind. Selbst die Weberknechte (Opilionidea) und die Milben (Acarina) haben an der Trümmerfauna keinen nennenswerten Anteil, obwohl sie doch sonst in der Stadt gut vertreten sind. Die nicht allzu häufigen Webespinnen (Araneina) stellen ein Assoziationsgemisch aus Keller- und Wohnraumformen, aber auch solchen des Gartens, der Wiese und der Felder dar, für deren Vorkommen wahrscheinlich ein gutes Ausbreitungsvermögen verantwortlich zu machen ist, da sie als Luftplankton über weite Strecken getragen werden.

Von Vertebraten haben nur Vögel und Säugetiere die Braunschweigische Trümmerfauna allgemein besiedelt. Zu nennen sind von Aves der auf den zerbombten Kirchen der Trümmerzone und der Schloßruine nistende Turmfalke, *Falco tinnunculus* L., der auf hohen Straßenbäumen und in Gärten sitzende Waldkauz, *Strix aluco* L., ferner Mauersegler, *Micropus apus* (L.), und Dohle, *Coloeus monedula* L., die beide in den zerbombten Kirchen der Trümmerzone nisten, außerdem einige im Trümmergebiet brütende Arten, so Haubenlerche, *Galerida cristata* (L.), Steinschmätzer, *Oenanthe oenanthe* (L.), Hausrotschwanz, *Phoenicurus ochruros* (Gmelin), und Girlitz, *Serinus canaria* (L.). Von Mammalia gehören zur Trümmerfauna Igel, *Erinaceus europaeus* L., *Oryctolagus cuniculus* (L.), Waldmaus, *Apodemus sylvaticus* (L.), und Mauswiesel, *Mustela nivalis* L.; für den Rand der Trümmerzone kommt noch die Wanderratte, *Rattus norvegicus* (Berkenhout), hinzu.

Zu besprechen sind noch die zerstörten Keller des Trümmergebietes. Der Schutz, den sonst die darüberliegenden Stockwerke den Kellern eines Hauses bieten, ist bei den Ruinen im allgemeinen nicht mehr vorhanden; die gelegentlich aufliegenden Schuttmassen stellen nur einen geringen Ersatz dar. Außerdem sind Fenster und Türen meist nicht vorhanden, so daß die Unbilden der Witterung nur unvollkommen abgehalten werden, ganz abgesehen davon, daß die Erwärmung durch ein bewohntes Haus im Winter fortfällt. Die Decke der Trümmerkeller ist in verschiedenem Ausmaß zerstört, so daß zwischen solchen Kellern, die vollständig unter Trümmerschutt begraben sind, und offenen Kellergruben, alle Übergänge bestehen. Die Fauna dieser Trümmerkeller unterscheidet sich von der Kellerfauna, die in alten Häusern Braunschweigs reichhaltig vertreten ist, recht erheblich. In der Trümmerzone sind charakteristische Glieder dieser Kellerfauna verschwunden, so beispielsweise die für das hiesige Gebiet als Glieder der Adventivfauna zu wertenden Nacktschnecken, *Limax* (*Limacus*) *flavus* L. und *Limax* (*Limax*) *maximus* L. Während die offenen Trümmerkeller in ihrer Fauna nicht wesentlich von den freiliegenden Trümmern abweichen, weisen Keller mit noch geschlossener Decke infolge ihrer meist größeren Feuchtigkeit gewisse Besonderheiten in der Fauna auf. Reich ist diese auf alle Fälle auch hier nicht, denn die zur Verfügung stehende Nahrung ist knapp. Selbst die Landasseln (Isopoda), die doch zur charakteristischen Kellerfauna gehören, sind in Trümmerkellern meist spärlich. In ihnen, die wegen der Einsturzgefahr vom Menschen meist gemieden werden, überwintern jedoch oft Mengen weiblicher Tiere der Stechmücke *Culex pipiens* L. sowie andere Mücken. Auch im Sommer bieten diese Keller zahlreichen Dipteren bei Wind und Regen Unterschlupf. Deshalb sammeln sich hier verhältnismäßig zahlreiche Spinnen (Arachnida) an, die sonst im Trümmergebiet Braunschweigs durchschnittlich selten sind.

Pflaster, Kellerböden, Höfe usw. sind der Anlaß, daß in den Trümmergebieten sich nicht selten Wasseransammlungen bilden, die mehr oder weniger lange fortbestehen. In gewissen Kellergruben Braunschweigs haben sich persistierende Wasserstellen gebildet, die sogar manchmal von Schilf und Binsen umwachsen und von

Wasserlinsen bedeckt sind. Es sind diese Wasseransammlungen Brutstätten für zahlreiche Mücken. Außer Larven von *Culex pipiens* L. fanden sich in großer Menge solche verschiedener Arten von Zuckmücken (Tendipedidae). *Anopheles*-Arten wurden in der Trümmerzone Braunschweigs nicht gefunden, obwohl ein indigener Herd dieser Mücken in dem Vorort Ölper vorhanden ist. In den Wasseransammlungen des Braunschweigischen Trümmergebietes fanden sich Kleinkrebse in Copepoda der Arten *Cyclops strenuus* Fischer und *Cyclops bicuspidatus* Claus häufig, stellenweise auch die Wasserassel *Asellus aquaticus* L. Wasserkäfer verschiedener Arten (Dytiscidae und Haliplidae) traten mehrfach auf. Auffallend ist, daß sich in solchen kleinen Wasserstellen verhältnismäßig rasch Populationen von Wasserschnecken gebildet haben, die nur passiv als Laich oder Jungschnecken eingeschleppt worden sein können; es handelt sich in Braunschweig um die Schlammschnecken (Lymnaeidae) *Radix peregra* (Müller) und *Radix auricularia* (L.) sowie um die bei uns durch die Aquarienliebhaberei eingeschleppte *Physa acuta* Draparnaud.

Es ist während des Krieges oft befürchtet worden, daß als Folge der Zerstörung der Städte eine Zunahme tierischer Schädlinge Schwierigkeiten mancher Art hervorrufen könnte, so beispielsweise verhängnisvolle Auswirkungen auf hygienischem Gebiet, vor allem durch eine Überhandnahme der Ratten. Erfreulicherweise sind derartige Schwierigkeiten nirgends entstanden.

Um zunächst auf die Arthropoden einzugehen, ist darauf hinzuweisen, daß die Stubenfliege, *Musca domestica* L., in der Trümmerzone Braunschweigs vollständig fehlt und auch sonst nichts über Anzeichen eines verstärkten Auftretens der Art in der Stadt oder gar eine Massenvermehrung während und nach dem Krieg bekannt geworden ist. Deshalb waren auch in hygienischer Beziehung keinerlei Schwierigkeiten durch diese Art zu erwarten. Nähere Verwandte der Stubenfliege kommen jedoch in der Trümmerzone vor, vor allem einige *Fannia*-Arten, aber auch *Hydrotaea*- und *Phaonia*-Arten; sie sind anscheinend nirgends verstärkt lästig geworden.

Auch *Culex pipiens* L. ist durch die umfangreichen Trümmergebiete nicht mehr als sonst im Stadtgebiet von Braunschweig aufgetreten, obwohl die überwinternden Weibchen in den zerstörten Kellern reichlich Unterschlupf finden; immerhin muß ein Fortbestehen dieser Refugien für überwinternde weibliche Stechmücken in sanitärer Hinsicht als bedenklich angesehen werden. Dasselbe gilt zum Teil auch für die kleinen Tümpel in der Trümmerzone als Lebensraum für die Entwicklung der Stechmückenlarven, wenn ihnen gegenüber der zahlreichen in Betracht kommenden anderen Gewässer im Stadtgebiet wohl auch keine allzu große Bedeutung zukommt. Daß *Anopheles*-Larven in der Trümmerzone nicht vorgekommen sind, wurde bereits erwähnt; davon, daß die Larven aber in der Großstadt gedeihen, habe ich mich früher in Berlin mehrfach überzeugen können.

Die Hausmaus, *Mus musculus* L., meidet die Trümmerflächen Braunschweigs weitgehend; sie wird dort meist durch die Waldmaus, *Apodemus sylvaticus* (L.), ersetzt. Die ursprünglich tropische, daher wärmebedürftige und an die Gebäude gebundene Hausratte, *Rattus rattus* (L.), die in Braunschweig vorkommt, findet sich nirgends in der Trümmerzone. Auffallend aber ist, daß auch weite Gebiete des Trümmerareals frei von der widerstandsfähigen Wanderratte, *Rattus norvegicus* (Berkenhout), sind, die bei uns im Freiland gut gedeiht. Das Ausbleiben von Rattenplagen führe ich darauf zurück, daß die Trümmergebiete den Ratten wohl ausgezeichnete Schlupfwinkel bei geringer Verfolgung bieten, doch meist unzureichende Ernährungsmöglichkeiten, nachdem einmal die Nahrungsvorräte in den zerbombten Häusern aufgezehrt waren. Allein in der Nähe der bewohnten Häuser bildeten sich am Rand der Trümmerzone in dieser Populationen der Wanderratte, die von ihren Schlupfwinkeln aus die bewohnten Häuser plünderten. Als später inmitten aufgebauter Viertel einzelne Trümmergrund-

stücke liegenblieben, siedelten sich dort bald Wanderratten an, die ihre Raubzüge in die benachbarten Häuser ausdehnten. Ihre Bekämpfung auf den unübersichtlichen Trümmern war manchmal nicht leicht. Erschwerend wirkte sich ferner der Umstand aus, daß nicht selten aus den Häusern Küchenabfälle und andere Dinge, deren man sich entledigen will, in nachlässiger Weise in die benachbarten Trümmergrundstücke geworfen werden, wodurch den Ratten stellenweise reichlich Nahrung geboten wird. Auch bieten solche Trümmerstätten manch anderen schädlichen und lästigen Tieren durch den Abfall bessere Ernährungsmöglichkeiten als die großen Trümmergebiete. Schon mit Rücksicht auf die Rattenbekämpfung ist das weitere Fortbestehen solcher Trümmergrundstücke bedenklich; sie sollten baldigst von Trümmern und Schutt befreit werden.

GESCHICHTE DER HAUSSCHÄDLINGSFORSCHUNG UND -BEKÄMPFUNG

HEINRICH KEMPER

Die tierischen Gesundheitsschädlinge, d. h. jene Tiere, die durch Stichbelästigung, Ruhestörung, Ekelerregung u. a. unser Wohlbefinden beeinträchtigen oder uns durch Krankheitsübertragung gefährlich werden können, teilen wir nach einem Vorschlag von Martini gewöhnlich ein in: Körperungeziefer oder Körpernister (die Läuse), Wohnungsnister (Bettwanzen, Flöhe, Schaben u. a.), Gemeindeungeziefer (d. s. die Arten, die nicht an die Wohnung, in ihrer Massenentwicklung aber an die Siedlung des Menschen gebunden sind, wie einige Musciden- und Culicidenarten), und schließlich das Freilandungeziefer (z. B. die *Aedes*-Mücken, Tabaniden, Heleiden, Ixodiden, Trombidiiden u. a.).

Diese nicht wirtsspezifischen Freilandnister waren in der Menschheitsgeschichte sicherlich die ersten, die als Plageerreger auftraten. Die stark spezialisierten Körpernister kamen später. Wohnungs- und Gemeindeungeziefer konnte sich natürlich erst dann breit machen, als der Mensch durch Viehhaltung und Ackerbau gezwungen wurde, immer wieder dieselbe Lagerstätte und Behausung zu benutzen, geschlossene Siedlungen zu gründen und Vorratswirtschaft zu betreiben. Seitdem hängt das Auftreten dieser Schädlinge von der Beschaffenheit dieser Wohnungen und Siedlungen ab.

In den ersten primitiven, wenig gegen Wind und Kälte geschützten Höhlen und Hütten, wo des Nachts immer die ganze Familie, oft die ganze Sippe und vielfach auch die Hunde, die jungen Lämmer und Kälber eng zusammengerückt schliefen, bot die Lagerstreu mehreren Schädlingsarten, vor allem den Flöhen, geradezu ideale Daseinsbedingungen.

Die Bettwanzen, die Schaben, die Heimchen, die Silberfischchen und was wir sonst heute zum Wohnungsungeziefer rechnen, stellen an die Höhe und Gleichmäßigkeit der Temperaturen höhere Anforderungen, als sie die ersten primitiven Behausungen des Menschen erfüllen konnten.

Die Bettwanze war den alten Griechen und Römern als Plageerreger bekannt. In Mitteleuropa ist sie aber erst im 12. Jahrhundert nachweisbar. Den Höhepunkt erreichte sie hier jedoch viel später, als man mehr und mehr dazu überging, die ganze Wohnung, also auch die Schlafräume zu beheizen. Wir hatten in den ersten vier Jahrzehnten dieses Jahrhunderts die stärksten Verwanzungen in den mit Zentralheizungen ausge-

statteten Mietskasernen der Großstädte. Nur zur Zeit des letzten Weltkrieges und in der ersten Nachkriegszeit war dieser typische Großstadtschädling in stärkerem Umfange auch in ländliche Gebiete eingedrungen, u. zw. in die hölzernen Wohnbaracken, die ja durchweg auch regelmäßig beheizt und dicht belegt waren.

Auch die Deutsche Schabe hat durch die Einführung der Zentralheizung profitiert, sie kann aber die damit verbundene zeitweilige Erniedrigung der Luftfeuchtigkeit im Gegensatz zu *Cimex* nicht gut vertragen. Deshalb beschränkt sich ihr Vorkommen weitgehend auf die Küchen, Baderäume, Heizungskeller u. a. Dafür bieten ihr die Heizrohrdurchtrittstellen und die Rohrverschalungen innerhalb der Häuser gute Ausbreitungs- und Versteckmöglichkeiten.

Für die verwandte Hausgrille, die „als Heimchen am Herde" zur Zeit unserer Großeltern eine alltägliche Erscheinung in den Häusern war, sind offenbar die modernen Wohnungen wenigstens in der Heizungsperiode zu trocken. Es gibt bei uns heute wohl ebenso viel, vielleicht noch mehr Exemplare dieser Spezies als früher, aber sie sind heute nicht mehr darauf angewiesen, im Innern der Häuser zu leben und zu überwintern. Wir dürfen sie eigentlich nicht mehr als Hausgrille, als Heimchen, d. h. Heimbewohnerin oder als *domesticus* bezeichnen. Sie kommen heute in Massen auf fast jedem Müllabladeplatz vor und können hier, wie auch auf manchem Komposthaufen, offenbar recht gut die kalte Jahreszeit überdauern. Bei uns sind sie neuerdings Gemeindeungeziefer, in den Subtropen waren sie schon immer Freilandnister.

Ebenfalls der veränderten Luftfeuchtigkeit wegen ist die Pelzmotte in Häusern heute kaum noch zu finden. Zur Zeit Réaumurs war sie die Motte, d. h. der Textilschädling schlechthin. Will man sie heute finden, muß man sie in Vogelnisthöhlen suchen. In den Wohnungen ist die weniger gegen vorübergehende Lufttrockenheit empfindliche Kleidermotte an ihre Stelle getreten.

Auch daß die Flohplage in den letzten 3 bis 5 Jahrzehnten so stark zurückgegangen ist, hängt sicherlich mit der gleichmäßigeren Beheizung und dem Trockenwerden der Wohnungen zusammen, allerdings auch noch mit manchem anderen, z. B. damit, daß sich die Kleidermode unserer Damenwelt sehr geändert hat, daß man die Strohsäcke als Schlafunterlage durch Sprungfedermatratzen ersetzte, daß Linoleum-Fußböden, Gebrauch von Staubsaugern und Bohnerwachs, Verschwinden der schweren Vorhänge und unnötigen Teppiche u. a. der Kehrichtansammlung entgegengewirkt haben.

Von den Wohnungsschädlingen, die es auf unsere Lebensmittel und Gebrauchsgegenstände abgesehen haben, sind durch den neuzeitlichen Lebensmittelverkehr und durch sonstige neuzeitliche Maßnahmen einige begünstigt, andere benachteiligt. Man kann wohl mit Sicherheit sagen, daß es vor 100 Jahren in den Wohnungen mehr Kornmotten, Speckkäfer, Fettzünsler, Mehlkäfer und Anobien, aber weniger Kornkäfer, Mehlmotten, Reismehlkäfer und Hausbockkäfer gegeben hat als heute.

Es wäre sicherlich interessant, einmal die Häufigkeit der einzelnen Hausschädlingsarten in den einzelnen Zivilisationsepochen eingehend zu diskutieren und die Abhängigkeit der Wohnungsschädlinge vom Lebensstandard des Menschen, vom veränderten Mikroklima in den Wohnungen und Siedlungen, vom Handel und Verkehr und von all dem, was man unter Namen wie Wohnkultur und Ortshygiene zusammenfaßt, darzulegen. Aber meine Aufgabe heute lautet ja anders. Ich soll sprechen über die Geschichte der Hausschädlingsforschung und -bekämpfung.

Eine wirkliche Forschung gibt es auf diesem Gebiete erst seit einigen Jahrzehnten. Natürlich hat man schon im Altertum und im Mittelalter an den Plageerregern gewisse Beobachtungen angestellt, und von diesen sind in den überlieferten Schriften auch manche mitgeteilt. Aber planmäßige Untersuchungen wurden kaum durchgeführt. Nach den Ursachen der Plagen fragte man meistens gar nicht. In besonders schweren

Fällen sah man die Kalamitäten als das Werk von Dämonen oder als eine von Gott verhängte Strafe an. Die führenden Köpfe, auch die Biologen, hielten noch bis gegen Ende des vorigen Jahrhunderts, also zu einer Zeit, als auf anderen naturwissenschaftlichen Gebieten schon großartige Erfolge erzielt waren, das Ungeziefer nicht für wichtig und würdig genug, als daß man sich ernstlich mit ihm befassen dürfte.

„Der Mann aus dem Volke", der immer wieder unter den Schädlingen zu leiden hatte, resignierte meistens. Und wenn er Bekämpfungs- oder Abwehrmittel anwandte, so waren das vielfach solche magischer Art. Bei den übrigen Mitteln handelte es sich um einfache Chemikalien und mehr noch um Stoffe pflanzlicher Herkunft, die aus der Volksüberlieferung stammten oder die in den Rezeptbüchern genannt waren. Die Autoren der mittelalterlichen Rezeptbücher hatten die Mittel auch nicht selbst erprobt, sondern sie meistens völlig kritiklos von Vorgängern oder vom Hörensagen übernommen.

Von den Mitteln, die z. T. schon im Altertum und bis ins vorige Jahrhundert besonders häufig empfohlen wurden, seien genannt: Wermut, Walnußblätter, Nieswurz, Koreander, Pfeffer, Farnkraut, Petersilie, Raute, Schierling, Knoblauch, Hanf, Bohnenblätter, Koloquinthen, alles dies meist als Abkochungen angewandt, und weiter Ochsengalle, Essig, Kochsalz (in Form von Heringslake), Ätzkalk, Kampfer, Quecksilber, Arsenik sowie Räucherungen mit Schwefel, Pech, Rinderhorn und getrockneten Haustierexkrementen. Wir sehen also, daß man bevorzugt solche Stoffe verwendete, die in Geruch, Geschmack oder sonstwie „scharf" waren oder deren toxische Wirkung auf den Menschen man empirisch kennen gelernt hatte.

Die hier zu Tage tretende Auffassung, daß die scharf riechenden und schmeckenden Mittel besonders wirksam seien, ist im Volke auch heute noch nicht völlig verschwunden. Ich weiß von einer Herstellerfirma, die viel Mühe darauf verwendet hat, ihr gut wirksames Fliegenbekämpfungsmittel von dem etwas stechenden Geruch zu befreien, und nun, nachdem ihr dies ohne Verminderung der Toxizität gelungen war, feststellen mußte, daß sich das Präparat jetzt nicht mehr so gut wie vorher verkaufen ließ.

Bekämpfungsmittel, die schon vor dem ersten Weltkrieg mit brauchbarem Erfolg angewendet wurden und sicherlich auch rein empirisch gefunden worden waren, sind vor allem: Schwefeldioxydbegasung, Kieselfluornatrium (gegen Schaben seit 1896 angewandt), Terpentinöl (besonders gegen Bettwanzen, seit 1737) und von den Drogen das Pyrethrumpulver (seit Anfang des 19. Jahrhunderts in Europa in Gebrauch).

Den ersten Anstoß, sich ernstlich mit gesundheitsschädlichen Insekten zu befassen, erhielt die Forschung durch die Entdeckungen Robert Kochs und seiner Schule. Die Feststellung, daß die Infektionskrankheiten durch Mikroorganismen erregt und daß diese Erreger vielfach durch Insekten und andere Tiere von Mensch zu Mensch übertragen werden, war wohl die segensreichste, die in der Geschichte der Menschheit je gemacht wurde. Aber es dauerte dann noch recht lange, bis man die Konsequenz zog und nun die Biologie der Überträger sorgfältig und planmäßig untersuchte mit dem Ziel, Mittel und Wege zur rationellen Bekämpfung auf breiter Basis ausfindig zu machen.

Diese Aufgabe wurde in größerem Umfange erst während des ersten Weltkrieges in Angriff genommen, u. zw. in Deutschland durch Männer wie Albrecht Hase, Erich Martini und Wilhelm Nöller. Die Not der Zeit war auch hier die Lehrmeisterin. Man wußte ja jetzt und fand es grauenhaft bestätigt, daß manchen Schädlingsarten eine ungeheuerliche Bedeutung für die Volksgesundheit und — in der damaligen Hungerzeit besonders — auch für die Volksernährung zukommt. Und es setzte sich jetzt erstmalig die Erkenntnis durch, daß die Schädlingsbekämpfung nicht Sache des einzelnen, jeweils Betroffenen, sondern eine öffentliche Angelegenheit ist.

Dieser Tatsache Rechnung tragend wurden schon während und gleich nach dem ersten Weltkrieg die hierher gehörenden Probleme in staatlichen Forschungsanstalten bearbeitet. Um 1927 gründete mein Amtsvorgänger, Prof. Julius Wilhelmi, in der damaligen Landesanstalt für Wasser-, Boden- und Lufthygiene, Berlin-Dahlem, eine Zoologische Abteilung, die sich seither fast ausschließlich mit den Gesundheits- und Wohnungsschädlingen forschend und beratend befaßt hat.

Es wurden von hier aus, und auch von anderen Forschungsstellen, Merkblattserien herausgegeben, Unterweisungskurse für Bekämpfungspraktiker, Amtsärzte, Kommunalbeamte u. a. durchgeführt, Auskünfte erteilt und vor allem gutachtliche Mittelprüfungen vorgenommen, um eine Sanierung des Bekämpfungsmittelmarktes zu erreichen. Wir können also feststellen, daß die Haus- und Gesundheitsschädlinge, obwohl sie lange vor den Pflanzenschädlingen für den Menschen von Bedeutung waren, doch erst später als diese bei den Naturwissenschaftlern Interesse und Beachtung fanden. Eine wissenschaftliche Forstentomologie hatten wir in Deutschland ja schon seit Beginn des vorigen Jahrhunderts, und die landwirtschaftliche Schädlingskunde wurde planmäßig und auf breiter Basis seit der Jahrhundertwende (1905: Gründung der Biologischen Reichsanstalt) studiert. Mit Haus-, Gesundheits- und Vorratsschädlingen hatten sich bis zum ersten Weltkrieg nur gelegentlich einige Mediziner, Pharmazeuten u. a. befaßt.

Von 1925 ab baute sich nach und nach eine Bekämpfungsmittelindustrie auf, die immer leistungsfähiger und zuverlässiger wurde. Und es wurden bald auch wirksamere oder sonstwie bessere Bekämpfungsmittel gefunden. Die Blausäurebegasung war schon gegen Ende des ersten Weltkrieges bis zur Praxisreife entwickelt. Später folgten — mehr für Anwendung im Kleinen (Einzelraumbegasung) — das Äthylenoxyd („T-Gas", seit 1932) und das Trichlorazetonitril („Tritox", seit 1939). Sehr erfolgreich war die Kombination des spezifischen Insektengiftes Pyrethrum (später auch des 1919 bekannt gewordenen Rotenon) mit stark benetzungsfähigen Erdölfraktionen. Das erste und bekannteste Handelspräparat dieser Art war das „Flit", das m. W. 1926 in den Handel kam.

Die Früchte all der damaligen Bemühungen konnten wir ernten im zweiten Weltkrieg und in den ersten Nachkriegsjahren, als manche Schädlingsarten und gerade die gefährlichsten unter ihnen stark an Häufigkeit zugenommen hatten. Hätten uns in den Jahren 1944, 1945, 1946 und auch noch 1947 nicht bessere Mittel und bessere biologische Kenntnisse als 20 Jahre vorher zur Verfügung gestanden, so wäre es nach meiner Überzeugung bei dem damaligen allgemeinen Chaos, dem Vertriebenenelend und der Hungersnot nicht nur zu schweren Schädlingsplagen, sondern zu verheerenden Seuchenkatastrophen durch die Schädlinge gekommen. Erfreulicherweise waren sich alle Verantwortlichen — unter den Siegern und den Besiegten — einig in ihrem Bemühen, mit allen, wenn auch manchmal nur mit sehr behelfsmäßigen, aber biologisch richtigen Mitteln gegen die Schädlinge vorzugehen. So konnte die drohende und nur von den Eingeweihten richtig erkannte Gefahr von Fleckfieber-, Malaria- und anderen Massenerkrankungen in Mitteleuropa noch gerade rechtzeitig gebannt werden.

Schon zu Anfang des zweiten Weltkrieges war, wie Sie wissen, auf dem Schädlingsbekämpfungsgebiet ein sehr wesentlicher Fortschritt erzielt worden durch die Entdeckung der insektiziden Wirkung des DDT, dem dann bald andere synthetische Kontaktinsektizide folgten.

Gleichsam damit die Bäume nicht in den Himmel wachsen, tauchte dann das Resistenzproblem auf, das ja gerade im Kampf gegen die Gesundheits- und Wohnungsschädlinge besondere Bedeutung hat. Es macht uns einigen Kummer, aber wir dürfen dabei die tatsächlich erzielten Fortschritte nicht übersehen. Wir erleben es heute,

daß der Mensch in seinem jahrtausendelangen, unendlich mühsamen und oft so hoffnungslos erscheinenden Kampf gegen die Schädlinge auf weiten Strecken schon gesiegt hat und gute Aussicht hat, überall den Kampf gegen die Insektenplagen endgültig zu gewinnen. Was es heute noch zu tun gilt, welche Probleme jetzt im Vordergrund stehen, das wird ja sicherlich im Laufe dieses Symposiums noch erörtert werden.

Lassen Sie mich bitte noch einige Worte sagen über den Beruf der Bekämpfungspraktiker: Sicherlich hatten schon im alten Ägypten und im alten Rom die reichen Leute besondere Sklaven angestellt und mit der Aufgabe betraut, für Ungezieferfreiheit in den herrschaftlichen Räumen zu sorgen.

Vom Mittelalter und vom Beginn der Neuzeit her sind uns die berufsmäßigen Rattenfänger bekannt. Sie standen wohl immer in höfischen Diensten und genossen z. T. hohes Ansehen. Der später viel verwendete Name Kammerjäger tauchte erstmalig 1649 auf, u. zw. in einem Scherzgedicht von Lauremberg. Dort wird er als modische Großsprecherei gerügt.

Wie ich vor Jahren einmal ausgeführt habe, scheint mir der Name entstanden zu sein aus einer Kontamination von Kammerdiener und Leibjäger. Es handelte sich wahrscheinlich um herrschaftliche Diener, die im Jagdgebiet das Raubzeug kurz zu halten und in den Wohn-, Speicher- und Stallräumen Ratten, Mäuse und andere Ungezieferarten zu bekämpfen hatten. Als „Ungeziefer“ galt ja alles, was nicht Nutzvieh war und nicht zur hohen Jagd gehörte, also auch Wildkaninchen, Füchse und Marder. Ursprünglich war „Ungeziefer“ die Sammelbezeichnung für alle Tiere, die als unrein galten, d. h. die nicht würdig waren, den Göttern geopfert zu werden.

Mit dem Ende der Feudalherrschaft wurde die Kammerjägerei zu einem völlig freien Gewerbe. Ihre Ausübung bedurfte — anders als beim Handwerk — keiner Genehmigung und keinerlei Ausbildung.

So kam es, daß das zeitweilig recht lukrative Gewerbe vielfach Leute anzog, die in anderen Berufen gestrauchelt waren. Die älteren von uns wissen noch, daß die Kammerjäger in ihren Reihen einen hohen Prozentsatz von Pfuschern und Betrügern mit langen Vorstrafenregistern hatten.

Besonders bedenklich wurde das, als diese Leute in steigendem Maße dazu übergingen, starke Gifte anzuwenden, deren Gefährlichkeit sie mangels Ausbildung gar nicht abzuschätzen vermochten.

Als der erste Weltkrieg die gesundheitliche und wirtschaftliche Bedeutung der Schädlingsbekämpfung so eindringlich vor Augen geführt hatte, wurde — nicht nur bei den an leitender Stelle Stehenden, sondern auch in der breiten Öffentlichkeit — der Ruf nach einer Sanierung des schlecht beleumundeten Kammerjäger-Gewerbes immer lauter.

Dieser Ruf kam einmal aus den Reihen der Kammerjäger selbst. Die Verantwortungsbewußten unter ihnen schlossen sich mehr und mehr zu Innungen zusammen, bemühten sich, die unlauteren Elemente aus ihren Reihen auszuschalten und sich fachlich ausbilden und fortbilden zu lassen. Sie strebten schon sehr frühzeitig danach, eine amtliche Anerkennung und Konzessionierung ihres Berufes zu erreichen.

Gleichzeitig bemühten sie sich, den Namen Kammerjäger durch einen besseren zu ersetzen. Nachdem Namen wie Zoologischer Desinfektor, Desinsektor, Entweser u. a., sich begreiflicherweise nicht hatten einbürgern lassen, setzte sich schließlich die Bezeichnung Schädlingsbekämpfer kurz vor dem zweiten Weltkrieg mehr und mehr durch.

Aber auch von oben her, von seiten der an diesen Fragen interessierten Wissenschaftler und Verwaltungsbeamten, wurde immer von neuem und zäh um eine Sanierung des so wichtigen Schädlingsbekämpferberufes gekämpft. Der Erfolg dieser Bemühungen trat nur ganz allmählich ein.

Es erwies sich als sehr schwierig, den Beruf richtig einzugruppieren. Er kann nicht eigentlich als Handwerk gelten, obwohl er viel mit diesem gemeinsam hat. Man konnte ihn nicht als völlig freies Gewerbe bestehen lassen, das hatten die schlimmen Erfahrungen gelehrt; man wollte ihm aber auch nicht alle Freizügigkeit nehmen, weil dadurch leicht die Initiative vermindert und der Fortschritt gehemmt wird. Der Gesetzgeber konnte sich nicht leicht dazu entschließen, wegen dieses im Vergleich zu anderen Sparten doch nur sehr kleinen Berufstandes ein kompliziertes neues Gesetzwerk zu schaffen.

Der entscheidende und volle Erfolg wurde in Deutschland erst 1948 erzielt, und zwar zuerst vom Senat West-Berlins. Dieser ließ durch sein Amt für Berufserziehung und Berufslenkung im Zusammenwirken mit den Fachwissenschaftlern die Unterlagen ausarbeiten und erließ dann eine sorgfältig durchdachte Verordnung zur Berufsregelung. Noch im gleichen Jahre wurden an die vorher gut ausgebildeten und sorgfältig geprüften Schädlingsbekämpfer die ersten Meister- und Lehrmeisterbriefe ausgegeben.

Dem Beispiel Berlins folgten bald die Bundesrepublik Deutschland und die Sowjetische Besatzungszone. Heute kann der Beruf als geordnet und gereinigt angesehen werden. Wir von der angewandten Zoologie haben dafür zu sorgen, daß sein Niveau nicht wieder absinkt, sondern noch gesteigert wird.

Der Schädlingsbekämpferberuf hat heute nicht mehr den Umfang wie in den dreißiger Jahren. Die Zahl der Gewerbebetriebe ist stark gesunken, ich schätze auf etwa ein Drittel. Das hängt damit zusammen, daß einige Schädlingsarten, z. B. die Bettwanze, die früher für die Praktiker das tägliche Brot bedeuteten, stark zurückgegangen sind und daß manche Bekämpfungsmittel, weil sie einfach und gefahrlos anzuwenden sind, bedenkenlos dem Laien in die Hand gegeben werden können.

Aber es werden sicherlich auch in Zukunft noch gewerbliche Schädlingsbekämpfer benötigt, vor allem dann, wenn es sich um schwierige und umfangreiche Plagen handelt. An diese Bekämpfungsfachleute wird man, was Können, Wissen, Wendigkeit und Zuverlässigkeit betrifft, in Zukunft mehr noch als in der Vergangenheit, sehr hohe Anforderungen stellen. Der Berufsstand darf nicht wieder zu einem refugium peccatorum, zu einem Sammelbecken Gestrauchelter werden. Dafür sind die ideellen und materiellen Werte, die er zu schützen hat, denn doch zu hoch.

THE ESTABLISHING OF IMPORTED STORAGE SPECIES IN THE ENVIRONS OF LARGE TOWNS

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The process by which an insect becomes an established pest in the buildings of a foreign country can be conveniently divided into three phases; 1) the invasion of buildings in its native area; to this can be coupled the gradual extension of its range in buildings, 2) its transport over long distances by ship or by land caravans, 3) its eventual establishment in a new area. The three phases are all subject to the same sort of controlling factors, but the last phase, the subject of this paper, cannot be discussed without some consideration of the others.

For many species, the process is complete and they are established in all the regions in which they can live and it is not easy to be sure which is their native area. Thanks to Dr. J. A. Freeman, a great deal is known about the spread of insects in ships, especially on the species, their abun-

dance and the products they attack. Very little, however, is known about the first phase and how insects become storage pests. In tropical areas, several do attack produce before it is harvested and are brought in with the crop. In drier areas, there may be outdoor storage, so there is no clear dividing line between outdoor and indoor insects; even in temperate areas, a few species may fly from building to building. But there are species now known only from man's storage buildings. These must presumably have existed and lived somewhere before man started to build and store. They may still exist out of doors and might be found if we knew where to collect.

A. CHARACTERISTICS OF STORAGE SPECIES

For an insect to succeed in living in stored produce, it needs the ability to eat dry foods and utilise and conserve the metabolic water produced. The absence of a specific feeding or oviposition behaviour pattern which might restrict the insect to some other ecological niche is also compulsory, e.g. many Bruchids cannot become store pests because they can only oviposit in growing seed pods. The kinds of insects able to enter storage premises easily are those which in their natural environment live on the kind of substances man stores, e.g. seeds, dried vegetable matter, including fruits, leaves and roots, dried meat, skins, wool and hair, and wood or something very similar to these substances.

Before man built stores, these insects probably utilised the smaller, but more widely spread stores of bees, ants, spiders, birds and rodents. There is another kind of insect of which a large number has also entered stores; those which are found scavenging in dead or rotting wood or under bark where they may have been eating fungi, frass, dead animals and preying on live ones. These have all benefited considerably from the more nutritious forms of food which are kept in a similar physical state in warehouses.

In conditions of regular shortage, some form of resting or resistant stage in an insect cycle is no doubt an advantage. This can be found in the cycle of several *Ptinus* spp. now found in bees' nests or in warehouses during the colder period of the year.

B. FACTORS AFFECTING INCREASE IN NUMBERS

1. Amount and time of storage

The original purpose of storage was to carry over food from one harvest to the next to ensure some food for the lean pre-harvest period. In primitive agriculture, this was probably seldom achieved but no doubt some food was available to pest species for a much longer period than in the natural environment.

For insects able to utilise stored produce, the most immediate advantage provided by man or storing animals is the quantity of food. The greater the amount of food, the bigger the population that can grow and develop on it without consuming it all and the greater the chance of some members surviving unfavourable conditions and being able to meet and mate afterwards.

Nowadays, vast quantities of food may be available and are often stored for long periods. This storage period enables pest insects to increase considerably in exporting countries and on snips so that large numbers are introduced into importing countries. Perhaps the increase on ships is becoming less significant as the speed of ships increases. When grain was carried on sailing ships, phenomenal increases in numbers were possible. Prolonged storage in the importing country has additional dangers because it enables the introduced species to recover from the disturbance of handling, to multiply whenever conditions are suitable, to invade the fabric of buildings, to spread to other produce perhaps more suitable for the species, to be carried away to other premises where the environment enables them to become established more quickly.

The two world wars of the twentieth century have been responsible for the spread and establishment of storage species, partly by necessitating prolonged storage in warm producing countries and partly by disrupting established trade routes and diverting pests to new areas. A considerable amount of storage was necessary during these wars in warm countries, so that insects increased both in numbers of individuals and species. In Australia for instance, *Rhizopertha dominica* has been numerous only during these two periods. This infested produce was often carried to unusual destinations, so that during these periods many species were introduced into new ports for the first time. The most spectacular introduction among these was that of the Indian khapra beetle of *Trogoderma granarium* Everts into Nigeria, S. & E. Africa and California.

2. Climatic influences

It is very evident in importing countries that some species which are imported regularly in large numbers fail to become established, often due to the climate. Failure can be caused in two ways; either the insect is actively killed by some unfavourable conditions, or although it can survive, it never gets sufficiently favourable conditions for it to increase. In Britain and northern Europe, some insect species are killed by cold winters, and others fail to breed during short or cool summers. Solomon and Adamson have analysed the survival of insects through the winter in Britain. The other aspect has not yet been summarised.

The effects of temperature and humidity on storage species can be easily studied in the laboratory and from laboratory results opinions can be formed on the likelihood of the species becoming established in a particular area. Climate, however, must be viewed critically. We have surprisingly few figures about the conditions in warehouses and usually have to work with summaries based on outdoor shade temperatures and with sparse humidity records. Buildings, even if they consist only of four walls and a roof, provide shelter and create a more equable and usually more favourable environment than is experienced in the open. Temperature, humidity and light will vary from place to place within the building and usually in temperate regions the mean temperature will be higher and in the tropics cooler than out of doors. Actual conditions will vary with the pitch, and material of the roof, the thickness and material of walls, presence of windows, the compass direction of the axes and the shape of the buildings, the numbers of storeys, presence of spaces, basements and attics, so that actual recordings of conditions are obviously needed for the particular building under study.

In addition many buildings liable to infestation are artificially heated. Some flour mills are kept warm at a fairly even temperature, provender mills may be warmed from the heat of machinery, maltings and bakeries are hot in places so that a whole range of tropical species can become established if they gain entry. The problem for the insects here is to travel from the ship or port warehouse to these heated premises. This has been made easy by the building of many of these buildings, especially flourmills and maltings, at ports and delivering imported produce straight to them. Buildings in inland towns are also infested. Sometimes this is because the insects themselves, if numerous, can create a favourable environment since they produce heat, and this enables them to increase rapidly and to resist external low temperatures. Chiefly it is because, in the past, produce was not inspected or treated if insects were seen.

It is worth noting here that our knowledge of the effects of the temperature and humidity are usually based on the complete life cycle of the insect, and hence our knowledge of resistance to unfavourable conditions rests on the least resilient stage—usually the young larva. The older larvae are usually much more resistant to unfavourable temperatures and humidities and since these conditions may slow up their growth, insects may survive some months in an unsuitable climate.

3. Foodstuffs

The rate at which insects increase varies with different foods and with the state of the food. This may be due to nutritive factors, occasionally to toxic factors as with the soy bean, and to the availability of food due to hardness or particle size. The humidity limits for species vary from food to food. Sometimes species compete with one another for a food, sometimes one species makes it more readily available to another. When residues are allowed to accumulate, populations may live in the fabric of buildings and there may be a succession of species as the character of the residue changes with break-down of old materials and addition of new food materials.

4. Dependence of rate of increase on biological characteristics

The intrinsic rate of increase is a theoretical value based upon the idea of a population of stable age. In practice this is seldom found in storage populations. An insect with a high intrinsic rate of increase will build up its numbers quickly and so gain the advantages associated with numbers. A high rate of increase is usually achieved by the rapid development of immature stages so that one generation succeeds another rapidly. This is very useful to the species especially when produce is not stored for long periods. A high oviposition rate, low developmental mortality, preponderance of females over males and long adult life are less effective in raising the rate of increase and need a longer storage period to take effect. On the other hand factors affecting these adversely can prevent increase of the species. Depression of the oviposition rate due to low humidity, or continuous light may not be obvious but be very effective in preventing a species from increasing. Resting stages lengthen the developmental cycle and check the population growth.

5. Association with buildings and disturbance

When infested produce is stored in a building the probability is that some of the produce will lodge in holes and crevices, and on ledges and that some of the insects will leave the produce and wander on to the walls, ceilings, beams and pillars and perhaps collect in crannies. This is often stimulated by the disturbance both of loading and discharging the produce, a procedure which also kills some of the insects. Some species also leave produce at a particular stage of the cycle, usually just prior to pupation. Many go no further than the sack in which the produce is packed or to the surface of bulk produce, but others spin cocoons in cracks in floors or walls or climb to the top of walls to pupate and are left behind when produce is removed.

Moths and some beetles fly, and some larvae walk about, especially at dusk or when produce is unloaded, and spread widely by so doing. In any event such species become established in buildings rather than associated with particular lots of produce. Insects from a warehouse may colonise the nests of many birds, rats and mice and sometimes of bees which may be built on or in storage buildings. Insects from nests may later reinfest the produce and occasionally may cause a new infestation if the bird acquires insects on some food or nest building material it obtains away from the warehouse.

6. Competition and mutual aid

Possibly the most difficult aspect of ecology to evaluate but certainly of importance in the establishing of a species in a new area is the interrelation of the various species in the environment. When species occupy similar niches they often compete and in laboratory cultures after a few generations, only one may be left. In warehouses the unsuccessful species may persist in very low numbers. For this kind of competition, a working hypothesis is to assume that the species with the higher intrinsic rate of

increase in the given physical environment will be the surviving one. The competition is more intense the more rapid the increase of each species. This assumption is not necessarily true, because the environmental conditions for which the rate is calculated and those to which it is applied differ, particularly as regards density, but the results of competition appear to bear out the hypothesis. Often it is not possible to determine the nature of the competition. The *Sitophilus* spp. for instance compete and one is eliminated but the mechanism of competition is obscure. Many storage species tend to be predatory, eating eggs, pupae, prepupae and larvae preparing to moult or recently moulted. A protective cocoon, or cryptic behaviour which shields these vulnerable stages, or a medium in which they are difficult to find assists the prey species to escape predation. In a medium such as flour at whose surface the vulnerable stages of prey species tend to collect favours the more predatory species and indirectly the protected species. Certainly the outcome of competition changes with the food as well as with the climatic conditions.

Often the presence of one species helps another to establish itself. For example a primary attacker makes it easier for a secondary species to gain entry; it may produce fine particles on which another species feeds; it may cause heating and moisture changes so making the physical conditions suitable for an other species, or induce the growth of fungi for yet others to eat.

7. Summary of principles

Summing up the general principles it may be said that to become established in storage premises in a new area, a species must be introduced regularly or in large numbers or at a time when at least a few individuals can multiply. It must find a suitable climate, or be able to survive any unfavourable times and multiply later, or in an unsuitable climate it must find a favourable niche in special premises. Once introduced it must be able to persist in the premises when the food is used or removed, be able to overcome the resistance of the native pests and withstand that of later introductions.

Nowadays a pest often has two extra hazards to overcome, inspection and control measures. An important weapon against inspection is the possession of cryptic stages or behaviour. A larva which is not easily detected even when abundant as with *Sitophilus* and Bruchids is useful but better still is a larva with cryptic behaviour together with an inconspicuous short lived adult as in *Trogoderma*. Rapid invasion of the cracks of buildings by *Ptinus*, *Oryzaephilus* and *Trogoderma* assists these against fumigants and also against insecticide sprays.

C. CONSIDERATION OF VARIOUS SPECIES IN BRITAIN

One of the most important findings of a survey carried out in Britain in 1938 was the absence of serious pests from premises kept exclusively for home grown grain and their abundance where imported grain was kept. In Britain, in fact, almost all serious pests are introduced, and even when a species is native, as for example, *Cryptolestes ferrugineus* and *Ephestia elutella*, the warehouse populations were originally imported. Truly native infestations may be limited to the clothes and house moths, *Tinea* spp. *Tineola bisselliella*, *Hoffmanophila pseudospretella*, and *Endrosis lactella*, the carpet beetle *Anthrenus verbasci* and minor beetles such as *Ptinus fur*.

Importation of storage pests into Britain is still considerable and continuous. Many species which are not established can be found in ports and large towns. Establishment is often still a matter of opinion based on experience and the views expressed here may not be accepted by other workers in this field.

The species will be dealt with in groups related in some way.

1. Important pests of whole grain

These are all introduced. *Sitophilus granarius* is probably established being reasonably cold hardy and able to breed at fairly low temperatures. All the grain weevils readily raise the temperature of their grain habitat and so survive low ambient temperatures. Even so it is unlikely that either form of *S. oryzae* can establish itself for long. The small form is the more cold hardy but probably succumbs to British winters in the absence of heating.

Rhizopertha dominica and *Sitotroga cerealella* have not become established although the latter is fairly tolerant of low temperature, but *Trogoderma granarium* in spite of tropical origins can withstand British temperatures for long periods, and multiplies whenever it finds heated conditions. It has been introduced during recent years on barley from Iraq and Iran, on grain from India, on groundnuts from Nigeria, on maize products from East Africa and on rice products from Burma. It became established between the wars when considerable amounts of imported barley were imported for malting. In maltings and distilleries the method of storing malt round the hot kilns to keep it very dry had prevented attack by the pests known previously, but it provided an ideal environment for *Trogoderma granarium*.

Ephestia elutella is probably a native British species but it has been imported on a variety of produce especially dried fruits and tobacco, and the imported populations establish themselves without difficulty. It became numerous in the 1930's in dried fruit warehouses but was controlled by barge fumigation of fruit before storage and by spraying of warehouses. As a result of long war-time storage during 1914—18 and the 1940's it established itself as a major grain pest. It is cold hardy when in diapause, as many are during the winter. *Cryptolestes ferrugineus* is another species which is native to British woodlands but which is imported on a very wide range of produce from all over the world. These imported individuals have become established. *Oryzaephilus surinamensis* has also become established, right through to farm premises although the British temperatures are very close to its lower developmental threshold. It is very cold hardy. No other species of *Cryptolestes* is established in granaries although *C. turcicus* is cold hardy.

A few other species may be pests of grain. Of these *Tenebroides mauritanicus* has not become established, possibly because it is seldom imported in any numbers and is unable to multiply in British temperatures. *Tribolium castaneum* is imported regularly in large numbers but is susceptible to winter cold and unable to multiply in our summers. *Ephestia cautella* occasionally survives the winter but can hardly be termed established. *Plodia interpunctella* may be established isolated buildings in which summer temperatures rise enough to permit some breeding.

2. Flour mills

The preceding remarks apply to the granaries and silos of flour mills. In the mill machinery, however, higher temperatures are maintained, cold hardiness is unimportant and the determining factors for establishment are ecological—those that can best utilise flour and other wheat products and withstand competition survive. It is interesting to note that the four or five species which are established in mill machinery are seldom imported into the mill with purchased wheat. Those that are so imported only become established when some other factor, usually climatic, excludes one of the following typical flour mill species of the temperate zone—*Ephestia kuehniella*, *Gnathocerus cornutus*, *Tribolium confusum*, *Cryptolestes turcicus* and *C. capensis*. All have some tendency to predation. *C. turcicus* probably owes its presence to the tough cocoon it spins before pupation. *C. capensis* is very restricted, apparently to coarser breaks in which there is more protection.

3. Peas and beans

No storage Bruchid is established.

4. General warehouse species

Stegobium paniceum and nearly all imported Ptinids are established. All are cold hardy and the success of establishment depends mainly on the summer increase of the species. Consequently *Ptinus tectus* quickly established itself around 1900. All other introduced *Ptinus* spp. (*clavipes*, "*latro*", *pusillus*) appear to be restricted to one generation annually and are relatively scarce. The other genera do not complete each generation as quickly as *P. tectus*. Nevertheless *Mezium affine*, *Gibbium psylloides* and *Niptus hololeucus* became established over 100 years ago, *Trigonogenius globulus* over 50 years ago and *Pseudeurostus hilleri*, only 20 years ago, and maintain themselves at low levels of abundance, with local concentrations. Spider beetles are seldom found in imported cargoes but find the climate sufficiently suitable for small numbers of introduced specimens to become established. Therefore new introductions when found of species such as *P. villiger* or *P. raptor* are promptly eliminated. Spider beetle adults benefit greatly from drinking so the dampness of Britain must help them considerably.

5. Bakeries and central heating

The heat from fires and central heating ducts has permitted the establishment of *Monomorium pharaonis*, *Thermobia domestica* and some cockroaches, in bakeries, hospitals and such premises. *Iridomyrmex humilis* which was established has been eradicated.

6. Clothes moths and carpet beetles

Many of these may be native species, certainly the common clothes moths are all resident species well established. The distribution of the carpet beetle *Anthrenus verbasci* illustrates the effects of partial dependence on the pollen of flowers of storage species.

Anthrenocerus australis is known to be introduced in Britain and north Europe, and to be establishing itself.

7. Other interesting species

From time to time, new pests unaccountably appear in stored products. This can happen when a new area enters trade, a new product is introduced or the mode of handling changed—enforced long storage or the introduction of steam milling for instance. The species concerned are often unknown from any natural habitat. The most recent example of this is *Cryptolestes pusilloides* which was discovered in Britain about 1940. Only a very small number of museum specimens, all in the U.S.A. and all taken from stored produce have been found bearing an earlier date. After the war this species was first taken frequently in Brazilian rice, and a little later in produce from Australia, S. Africa and the Plate region. The species is not established anywhere in the northern hemisphere but appears to be spreading from South Africa into East Africa.

Aphomia gularis is established in a small number of premises handling edible nuts. In unheated premises it is confined to the south of England but it is also established in some heated manufacturing premises in the north. The Infestation Control Laboratory of the Ministry of Agriculture are noting the occurrence of this species and of *Anthrenocerus australis*. Because of its restricted food and because it is at the limit of its climatic range, it should be easy to eradicate *A. gularis* if desired. On the other hand, it is probable that *Anthrenocerus* will continue to spread.

In conclusion it can be said that it should be possible from a thorough knowledge of storage species to explain why a particular species has or has not become established in any area of the world. Also it is possible to predict reasonably accurately the chances of any new introduction becoming established, and more important to draw attention to the circumstances which will assist it to do so.

DISCUSSION

R. OESER: What is your meaning about the importance of aviation considering the introduction of pest insects?

R. W. HOWE: In answer to the question about introduction of insects by air by Dr. Oeser. This is a real risk and produce moved by air should be carefully inspected and treated. However, produce moved by air would certainly be valuable and would stand the cost of treatment. Foodstuffs liable to heavy infestation are not carried by air at present.

Introduced insects also must be able to compete with the resident forms of they are to become established.

I think that the factor that controls the abundance of *Niptus hololeucus* is the presence of water for drinking by the adult which greatly improves egglaying. If a connection can be established, as it probably can, between damage and dampness it would help to explain the abundance of *Niptus* in these towns.

SYMPOSIUM XI

INSECTICIDE RESISTANCE

INSECTICIDE-RESISTANCE AS A PUBLIC-HEALTH PROBLEM AND ITS IMPLICATIONS

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This contribution will be in the nature of a progress report, since the subject of resistance in insects of public-health importance has been so frequently reviewed. Possibly the simplest course is to project slides tabulating species, places and dates of first appearance of resistance, and to discuss only the new instances since the last International Congress in 1956.

It is now clear that the housefly can develope resistance in any part of the world, and reports of DDT-resistance have now been made from 2 countries that formerly disclaimed it, namely China and Czechoslovakia. *Musca nebulo* in India has shown decided BHC-resistance in Assam and Bombay, but its level of DDT-resistance in treated areas is not high. Resistance to diazinon, first noted in Denmark, has developed in Switzerland, Italy and New Jersey; resistance to malathion, first observed in Florida, has now been reported from Georgia, Arizona, Louisiana, and is probably present in California. In Georgia it was found that certain populations had developed the characteristic of avoiding malathion baits.

Resistance to dieldrin and heptachlor has developed in the *Culicoides* sandflies of coastal Florida. A most serious development is the appearance of dieldrin-resistance in the sheep blowfly in New South Wales and at certain other points in Australia; it has also been reported from South Africa, and from New Zealand in the related species *P. sericata.*

DDT-resistance in body-lice has now appeared in Yugoslavia, where formerly it had been reported not to have developed, and there are new foci of resistance in Asia, Africa and America. The development of BHC-resistance in South and West Africa has been confirmed, and it has also appeared in Iran. The cross-resistance to dieldrin is much stronger than to BHC, and a decisive dieldrin-resistance has been found in body lice in Tanganyika. Chlordane-resistance of the German roach has now developed in parts of Central America and the Caribbean area, and in Canada. A definite resistance to DDT has been reported from western Europe, where this insecticide has been used for roach control. There have been 2 reports of malathion-resistance, but they are not adequately supported by test figures; however, resistance to malathion has been developed by pressure on 2 laboratory strains. A diazinon-resistant strain has recently been discovered in Kentucky, and more are suspected. DDT-resistant populations of the bedbug have evidently been encountered in eastern Europe, and BHC-resistance has

been reported from Israel. The tropical bedbug *Cimex hemipterus* has now developed DDT-resistance in parts of East Africa and in Trinidad; dieldrin-resistance has appeared in East and West Africa and in Malaya.

Although we have heard no more of resistance in the human flea, populations of *Ctenocephalides* fleas have been reported resistant to DDT and BHC in Hawaii, and to BHC in Hong Kong and Japan. Proven DDT-resistance has at last appeared in the oriental rat flea, in a part of Bombay state which has received 2 annual sprays of DDT for the last 14 years.

Resistance to BHC and toxaphene has now been reported for certain strains of the blue tick in Northern Rhodesia. Resistance to DDT, which had been substituted for BHC in places in South Africa and Australia, has now been fully documented for the blue tick and cattle tick. Chlordane-resistance of the brown dog tick has been recently reported from Panama and Texas. Control failures with BHC and toxaphene on cattle lice (*Bovicola caprae* and *B. limbata*) have also been reported from Texas.

Passing to mosquitoes, we find that the tropical house mosquito is evidently going the way of the housefly. Increased DDT-resistance and a high resistance to BHC and dieldrin is the usual story wherever these chlorinated hydrocarbons have been used, with the noteworthy exception of the southeastern U.S. and California. A malathion-resistant strain has recently been discovered at Duala, Cameroun. DDT-resistance has also been noted in *Culex coronata* in Panama and dieldrin-resistance in 3 species of *Culex* in West Africa. Dieldrin-resistant *C. pipiens* have now been reported from southeastern France and are suspected to occur in Massachusetts, while in Japan there are strains of *C. pipiens pallens* resistant to both classes of chlorinated hydrocarbons. *Culex tarsalis* has not moved much in the last 4 years, although there is evidence of developing dieldrin-resistance in Utah and Oregon. The OP-resistance of this species in Fresno county, California, has proved to be restricted to malathion.

DDT-resistant strains of the yellow fever mosquito continue to appear in the Caribbean area, recent records coming from northeastern Colombia, Haiti, Guadeloupe and Jamaica, besides French Guiana, recalling an early unconfirmed report from Dutch Guiana. Increased DDT-tolerance of adults has been reported from Saigon in South Viet Nam. A dieldrin-resistant strain with a curious cross-resistance to DDT has appeared at San Juan airport, Puerto Rico. The same type of resistance has been recently noted in *Ae. taeniorhynchus* on Cockspur Island, Georgia. The only recent record for salt-marsh mosquitoes is that of BHC-resistance in *Ae. sollicitans* in Delaware. The increase in OP-tolerance observed by test in Florida has not been sufficient to prevent control by malathion. Among the irrigation-water mosquitoes, *Ae. nigromaculis* has developed parathion-resistance in Kings and Tulare counties, California; although the increase in LC_{50} was only 2—3 times the normal, it was sufficient to abolish control.

Meanwhile in the anopheline mosquitoes, the vectors of malaria, the resistance problem has been growing to serious proportions. In 1956 only 5 species were involved: DDT-resistance in *Anopheles sacharovi* in Greece, *A. sundaicus* on the north coast of Java, and *A. stephensi* in Saudi Arabia; and dieldrin-resistance in *A. gambiae* in northern Nigeria, *A. quadrimaculatus* in Mississippi, besides *A. sacharovi* in Greece. Now resistance has developed in 21 anopheline species, of which 7 show DDT-resistance and no less than 20 species have populations with dieldrin-resistance. The only species to have developed only DDT-resistant populations is *A. sundaicus*. Yet dieldrin is a newcomer compared to DDT. Species like *A. pseudopunctipennis* in Mexico, and *A. fluviatilis* and *A. culicifacies* in the Middle East and India, had withstood over 10 consecutive years of DDT pressure without losing their susceptibility, but dieldrin-resistance developed in places after just one or two applications. The dieldrin-resistance of *A. gambiae* has

spread to 7 countries of West Africa, sometimes in the interior and sometimes on the coast. Dieldrin-resistance has developed in *A. albimanus* in 5 Central American states, 3 Caribbean regions, Mexico and Ecuador. *A. aquasalis* and *A. albitarsis* are two other American species to go dieldrin-resistant. *A. labranchiae* in Morocco, *A. pharoensis* and *A. sergenti* in Egypt and Palestine, *A. coustani* and *A. pulcherrimus* in Saudi Arabia, *A. subpictus*, *A. barbirostris* and *A. annularis* in Java, and *A. minimus* and *A. vagus* in the Philippines, complete the list of dieldrin-resistant species in the Old World.

New records of DDT-resistance include *A. subpictus* in the northern half of the Indian subcontinent, *A. albimanus* in 4 Central American States, *A. quadrimaculatus* in 2 of the United States and in Mexico, and *A. pharoensis* in Egypt. DDT-resistance of *A. sacharovi* has been found in Iran and especially southern Turkey, of *A. sundaicus* has been found in Burma; while that of *A. stephensi* has been found in all the countries around the Persian Gulf. The recent discovery of dieldrin-resistance as well in *A. stephensi* at the mouth of the Persian Gulf has posed a formidable problem in the control of this serious malaria vector. The use of organophosphorus compounds as remedial insecticides is being widely tested but no single compound has yet been moved into general practice. In all, resistance has developed in anophelines in so many parts of the world that the combined areas contain between 5 and 10 per cent of the human populations covered by the global malaria eradication programme.

Our detailed knowledge of resistance in anophelines has been achieved by the use of standard test methods for adult mosquitoes and mosquito larvae. The World Health Organization was chosen as the authority behind their standardization, and many of those present here, especially our chairman, have participated in their development. Several hundred test kits have been distributed to public health organizations, and their use is being pressed strongly. A standard test method is also available for body lice, and there are provisional methods for bedbugs, fleas, *Phlebotomus* sandflies and blackfly larvae. A method is being developed for testing the irritability to DDT of adult anophelines, in an attempt to gain some quantitative data on the vexed question of behavioristic resistance. Use of the test kits has given the explanation of control failures which might otherwise have been blamed on faulty application, and occasionally they have revealed resistance where it was not suspected. Their success has prompted the Entomological Society of America to set up a committee on test methods for agricultural insects. The provisional method for *Phlebotomus* showed that DDT-resistance has not developed in this genus in Greece. *Glossina*, Triatomines and Simuliids represent the other insect vector groups in which resistance has not developed. Meanwhile among the acarines it is noteworthy that BHC-resistance has not yet been reported in *Ornithodoros* ticks.

The symposium chairman has requested that something be said about "resistance hazard", why some species and genera tend to develope resistance more than others and why it seems to be more common among pests of public health importance. The box score for resistance among agricultural species of arthropods is now 61, as compared with 62 for medical and veterinary species. As a whole the resistance picture, whether in agriculture or in public health, is correlated with the total amount of insecticide applied, both in geographical extent and in the number of years applied. The rate of development is *a priori* proportional to the intensity of selection; larval selection will operate against both sexes and adult selection will hit the males more heavily, as Hoskins has pointed out. Although no correlation could be found by Busvine between the number of generations per year and the rate of development of resistance, there is no doubt that in important North American species the problem is much farther advanced in multivoltine U.S. populations than in univoltine Canadian ones. Treated populations which are not genetically diluted by untreated ones will develope resistance the faster.

Of course the origin of the resistance hazard lies in the genes, or rather the mutant alleles of the wild-type susceptible genes. If we can detect their presence and gauge their frequency, we can assess the resistance hazard of a species. The now classic example is *Anopheles gambiae*, where a test with impregnated papers will reveal the individuals heterozygous and those homozygous for the dieldrin-resistant gene. Extensive testing has shown heterozygotes to exist in untreated populations in the interior of West Africa, whereas the gene is apparently absent in East Africa from Sudan down to Rhodesia. However, the samples tested from the field should be as large as possible, and even then may be of inadequate size if the frequency of the mutant allele in the population is low, say 1 in 20,000, that for homozygotes for the albinism gene in humans, which is quite a usual figure for mutant frequencies. Certainly exposing laboratory populations from limited foundation stock to selection pressure is the least sensitive method for testing for resistance hazard. Experience with a species in one part of the world may lead to a feeling of security which may be belied elsewhere; *Stomoxys calcitrans* and *Fannia canicularis* did not develope DDT-resistance in North American barns, but DDT-resistant populations of *Stomoxys* developed in Scandinavia and of *Fannia* in northern Spain. Unfortunately it is the general experience, especially in agricultural insects, that serious resistance problems do first appear as isolated foci within a complaisant overall situation.

Even when the genetics of the particular resistance has been worked out as it has in *A. gambiae*, it is nevertheless possible with the standard test methods at multiple concentrations to assess the resistance hazard. If the sample tested shows a plateau at the top of the dosage-mortality line, a few individuals much more resistant than the rest are indicated. If the source of their resistance is monogenic, and in this case it probably is, succeeding generations under pressure may be expected to show regression lines that are not only farther to the right, but also flatter or with a lower plateau. On the other hand the original sample may show a d-m line that remains straight to the top, which in successive generations under pressure moves gradually to the right without material change in slope. This may indicate a vigor tolerance due to multiple, non-specific genes making for a stronger constitution generally but not for absolute resistance to the insecticide. Vigor tolerance may not pass the point where an increase in dosage fails to control, as was found with the DDT-tolerant larvae of *An. quadrimaculatus* in Alabama, and is being now found with the DDT-tolerant adults of *An. sacharovi* in Greece. The DDT-tolerant *An. albimanus* in Central America may be controlled by heavy deposits of DDT, so that the point now arises whether this is a specific DDT-resistance or a cross-resistant manifestation of prevailing strong dieldrin-resistance in the region.

Although dieldrin-resistance in *An. gambiae* and certain other mosquitoes, in the housefly, German roach, bedbugs, ticks, fleas and many agricultural insects shows no cross-resistance to DDT, there is recent evidence of the occurrence of a type of dieldrin-resistance which extends to DDT. First reported for *Aedes taeniorhynchus* in Georgia, it is also present in a population of *Aedes aegypti* in Puerto Rico. Repeated backcrossing of this latter strain in our own laboratory with a normal strain, while maintaining either DDT or dieldrin pressure, has revealed that the DDT-resistance is inseparable from the dieldrin-resistance, and that the characteristics are inherited as a single entity linked with Chromosome 2 of this species, at a crossover distance of 25 units from the marker gene yellow.

The characteristic of true specific DDT-resistance in the housefly has been shown by the groups of Kearns and of Hoskins to be a high content of DDT-dehydrochlorinase enzyme detoxifying DDT to DDE. The single gene for DDT-resistance characterized for the strains of NAIDM origin by Lichtwardt, and the content of the enzyme in

individual flies, have been shown by Lovell and Kearns to be inherited in parallel; heterozygous hybrids have half as much DDT-dehydrochlorinase as the resistant homozygotes, as if the mutant gene allele developed so much enzyme. The single gene shown responsible for DDT-resistance in *An. sundaicus* may have a similar effect, since dehydrochlorinating ability has been demonstrated in vitro for the resistant strain of this species. DDT-resistant strains of *An. sacharovi* and *An. atroparvus* produce much more or slightly more DDE, depending on the dosage, than susceptible strains; but the results were obtained only in vivo and it is uncertain whether the extra DDE production is a cause or effect of the DDT-resistance. Although a single gene is evidently responsible for DDT-resistance in the Trinidad strain of *Aedes aegypti*, dehydrochlorinating ability cannot at present be demonstrated in vitro; comparison of several resistant with susceptible strains, and within a strain in which resistance is varied by selection, leaves the same uncertainty whether DDE production is responsible for DDT-resistance in *Aedes aegypti*.

Indeed, it is uncertain whether DDT-resistance in all strains of *Aedes aegypti* is due to the same gene; that in Malayan strains is probably different from that in Caribbean populations, while a DDT-resistance inducible by malathion pressure may be different and that of the Puerto Rico strain is almost certainly something else again. One understands that in the housefly Milani has evidence for 3 different autosomal genes for DDT-resistance, while Kerr has found a DDT-resistant gene on the Y-chromosome. On the other hand, certain genes for resistance may be the same in more than one species, as Oshima has found the same genes for DDT-resistance and for nicotine-resistance to be present in *Drosophila virilis* as well as *D. melanogaster*.

It is the existence of such genes in small fragments of natural populations that decides the resistance potential of species. The role of research is to characterize these genes not only by their mode of inheritance, but also by their cross-resistance pattern and finally their biochemical peculiarities. For example, we have in our laboratory malathion-resistant strains of *Culex tarsalis* and of *Aedes aegypti*. The former is specific to malathion, with no cross-resistance even to other OP compounds, and is characterized by increased carboxyesterase activity. The latter is cross-resistant to Sevin and DDT, inter alia, and is characterized by an increased phosphatase activity. Many years ago Busvine distinguished between the DDT-resistance of a Sardinian and an Italian (Torre in Pietra) strain of houseflies in that the latter had a peculiar cross-tolerance to pyrethrins. The same pyrethrin-tolerance was present in the early days of the Orlando strain of houseflies, and has recently been observed in a DDT-resistant strain of *Cimex hemipterus* at Mombasa, and in DDT-resistant blue ticks in South Africa. Perhaps this DDT-resistance then is a different type biochemically and genetically.

The full understanding of these resistance entities is important if we are hopeful of the search for negatively-correlated compounds. For example, negative correlation has been reported between malathion and DDT for one strain of houseflies in Sicily and between malathion and dieldrin for a population of the cotton boll weevil. Negative correlation has been detected between DDT and diazinon not with regard to survival but for subsequent fecundity. Disappointments have been encountered with DTP and CBA for DDT-resistant houseflies. The only negative correlation that is sound on a genetical basis is that between PTU and DDT in *Drosophila*, because it is tied to the same gene and is therefore inseparable by the genetic recombination constantly taking place.

There is now evidence of negative correlation between diazinon and PTU or phenyl isothiocyanate in houseflies. Whether the negative correlation between Sevin and DDT, observed in the Korean strain of the body louse, is truly genetic remains to be ascertained. It is clear that if the resistance hazard to established insecti-

cides reside in the genes, it is in the same genes that we must look for the weak spots for negatively correlated compounds.

It would appear that students of insecticide-resistance have reached the point where biochemistry and genetics must together be added to just plain horse sense to ensure understanding of this multiple phenomenon. It is not so much resistance but its investigation which is furthest advanced in insects of public-health importance, not only because they are easier to rear than agricultural insects, but also because of the esprit-de-corps created by the emergency of the malaria eradication programme in particular and the action of WHO in general.

RESISTANCE OF AGRICULTURAL INSECTS TO INSECTICIDES

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An agricultural pest, *Quadraspidiotus perniciosus* was the first insect known to have developed resistance to an insecticide (Melander, 1914). By 1940 several scale insects and the codling moth had become resistant. The problem was important enough to prompt H. S. Smith (1941) to make it the subject of his Presidential Address to the American Association of Economic Entomologists. The development of resistance to DDT by *Musca* a few years later served as a warning that potent new insecticides could become as ineffective as those materials that had been replaced.

The many excellent reviews of the subject make it unnecessary to compile another summary at this time. Spiller (1958) has already called attention to the importance of the problem in agricultural insects. He has also summarized the work on the mechanisms of resistance obtained from study of insects of importance in public health. As he stated, there is little information of this sort in the literature on the agricultural insects. On the other hand, there are some observations of value, particularly in practical control of resistant insects.

Many agricultural insecticides were used regularly for decades without selection of resistant strains, or development of resistance. The oil sprays which replaced both lime-sulfur solution and HCN for controlling resistant scales, nicotine, pyrethrum and cryolite were effective materials with no instance of resistance following practical use. Thus, there is evidence that insects do not have mechanisms for defense against all chemicals toxic to them.

The development of resistance in agricultural insects in small areas has been noted by Way (1959). Melander (1914) found resistant scales in a small area in Washington. Within ten years resistant scales were common in most important deciduous fruit areas in the United States. Similarly, Hough (1928) demonstrated resistance of codling moth larvae from a small river valley in Colorado. Within 15 years resistance was a general problem, and resistant codling moths contributed to abandonment of apple growing in some areas. On the other hand, the Colorado potato beetle (*Leptinotarsa decemlineata*) developed resistance on Long Island (New York), where it is a serious

pest, as early as 1952 (Quinton, 1955). In Connecticut, only a few miles away, it is not a serious pest and has apparently not developed resistance. The potato flea beetle (*Epitrix cucumeris*) is a serious pest in Connecticut and not on Long Island. Kring (1955) found resistance to DDT in Connecticut, but no reports of resistance have come from Long Island. Kring's findings were questioned, and he verified them by obtaining flea beetles from Maine which proved to be much more susceptible to DDT than flea beetles from any part of Connecticut.

Thus, development of resistance in small areas is sometimes followed by a general development. When this does not happen, it is obvious that the insects do not react the same way in all parts of their range. This suggests that migration of resistant insects in agriculture may be restricted, and implies that the genetic constitution of a species may vary from area to area.

The practical solution for the first problem of resistance was substitution of oil sprays for lime-sulfur solution. This worked so well that no one found it necessary to investigate the San José scale problem further. Resistance in insects is still being met in the same way. Primitive as it may seem, it is the only method available, and will be successful as long as effective alternate materials can be developed. However, as Busvine (1959) has pointed out, the effective materials represent only two or three types of modes of action. There is obviously a serious need for insecticides of differing modes of action, and for all investigations which might lead to discovery of such materials. The chemical industry of Europe and the United States, which has produced so many effective materials, needs the active cooperation of entomologists in discovering new modes of action.

It may also be time to consider some "control" of the use of materials, preferably by manufacturers and distributors. An effective new material might be tested in the laboratory to determine whether or not it selected for resistance. If it did, its use could be restricted in a given area before it became ineffective. After two or three years it could be used again successfully. This alternation would seem applicable in control of insects affecting public health.

In the United States use of insecticides is being opposed by many people because of concern about residues and effects on animals other than insects. More recently the development of resistance has been used by the opposition, on the grounds that resistant insects cannot be controlled. Whether or not these arguments and concerns are valid, it is obvious that economic entomologists in the United States are being challenged to develop effective insect control without use of "poisons". To paraphrase the conclusion of Smith (1941), resistance continues to be an obstacle to the practical usefulness of entomology. The ingenuity and hard work of the past 20 years has not overcome the obstacle. Therefore, new approaches must be found and followed if damage and annoyance by insects is to be kept at a minimum.

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INSECTICIDE RESISTANCE AMONG PESTS OF STORED PRODUCTS

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Reports of Resistance in the Field

In recent years there have been occasional suggestions that failures in control operations against pests of stored food might be due to incipient resistance. Usually it has been difficult to exclude faulty control measures, since confirmation in the shape of resistance tests has seldom been attempted. However, there is some interesting evidence concerning possible resistance to gamma BHC by strains of *Tribolium castaneum* from Africa. Tests at the Cooper Technical Bureau, Berkhamsted, indicated low levels of BHC-resistance in strains from Nigeria and Sierra Leone, while a strain from Kenya was about 12 times as resistant as the laboratory colony (5). After 2 years' non-selective breeding, this strain was still 5 times as tolerant as the normal. Clearly, then, the reduced susceptibility was inherited; but it was not established whether specific resistance or vigour tolerance was involved.

Other field reports of possible resistance to BHC concern *Sitophilus oryzae* (10). However, the evidence is not conclusive and an extra complication is possible confusion with the recently separated species *S. sasakii*.

Generally speaking there is very little uncontrovertable evidence of resistance arising in the field among the beetles and moths which breed in stored products. Perhaps this is due to the particular circumstances of the infestations, which are usually replenished at intervals from external sources; so that any selected strain would tend to be diluted by insects from untreated populations.

Laboratory Investigations

In the interpretation of tests for resistance, it is obviously of interest to know what differences in susceptibility may be expected between different "normal" colonies. To this end, measurements of susceptibility to gamma BHC and pyrethrins have been made on 6 unselected laboratory colonies of *Tribolium castaneum* (4). Distinct differences were found, the weakest strain being 4 times as suceptible as the strongest. Parallel differences in suceptibility to pyrethrins were also found, which suggests that unspecific vigour tolerance, rather than true resistance, was involved.

In other laboratory investigations there have been attempts to induce resistance by repeated selection; these may be summarised as follows:—

Reference	Insecticide	Insect	No. of generations	Increased tolerance
(8)	DDT	*Sitophilus granaria*	7	slight
(7)	DDT	*Tribolium castaneum*	10—15	2 to 8
(10)	gamma BHC	*Sitophilus oryzae*	16	2.5
(1)	pyrethrins	*S. granaria*	15	3.5
(3)	HCN	*Tribolium confusum*	7	slight
(9)	Methyl bromide	*S. granaria*	14	2
(6)	Methyl formate	*T. confusum*	35	3
(2)	Mercury vapour	*S. granaria* (eggs)	10	350

The case of the resistance to mercury vapour by *S. granaria* eggs is rather different from the others in several ways. Selection was accidental and apparently due to traces of mercury in an incubator. Experiments with eggs of *S. granaria* colonies from various sources revealed large differences of susceptibility (over 55 times). It is clearly a very variable character, which the authors concerned ascribe to differences in permeability of the egg chorion to the mercury vapour.

Apart from this rather special case, the attempts to induce resistance to insecticides by stored product beetles have resulted in comparatively little loss of susceptibility. The amounts are of the order associated with vigour tolerance rather than true specific resistance.

In contrast to this, there have been some interesting developments when a field strain showing tolerance was further selected in the laboratory. A strain of *Sitophilus granaria*, taken from a warehouse in which several applications of synergised pyrethrum had been made, was found to have a tolerance 3.5 to 6.5 times that of a laboratory colony (4). This tolerant strain was colonised in the laboratory for 4 years, without selection, at the end of which time its resistance had fallen to about double that of the susceptible laboratory colony. Subsequently, it was sent to the D. S. I. R. Pest Infestation Laboratory and subjected to steady selection, by exposure to pyrethrins in 19 out of 24 generations, and it has now reached a level of 36 times the normal resistance (10, 11). It is not certain that the limit has been reached, but technical difficulties make it hard to measure levels much higher than this. It is interesting to note that resistance to synergised parethrins has only slightly increased (twice) during the period of selection. Work on this interesting colony is proceeding.

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PHYSIOLOGY AND BIOCHEMISTRY OF RESISTANCE TO CHLORINATED HYDROCARBONS

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Since resistance to insecticides first assumed importance in the case of cyanide on red scales in California in the early part of this century, there has been an ever increasing interest in the details of insect biochemistry and physiology. This is largely the result of a hope that if enough is known about both normal and insecticide-resistant insects, methods for overcoming the resistance will become apparent. On this basis, the universities and other research organizations, governmental agencies, and the agricultural chemical industry have supported almost any research project which might be interpreted to

bear on the subject even remotely. Vast sums of money and, more important, much research talent and facilities, have been put into the effort. Much has been learned about the details of insect life processes but to the present time the only practical way to control a species which has developed resistance to an insecticide is to use one of another chemical family which presumably acts in a different manner. And the members of the rather large arsenal of insecticides now available have been found almost entirely on the empirical basis of "Try everything".

In the more basic studies on resistance two rather distinct points of view may be discerned. On the one hand, a researcher who focuses his attention upon the resistance process(es) will be concerned with absorption, distribution and storage of the chemical in the insect body, its excretion, the reactions by which it is degraded to nontoxic derivatives and their disposal, in short, with the processes which affect the insecticide in any manner that diminishes the toxic effect. On the other hand, his attention may be given primarily to the toxic effects caused by the insecticide in the susceptible insects, to the disturbances of essential biological processes which result in the toxic effects and to the differences between susceptible and resistant strains in this respect. Thus the causes of resistance may be sought either in what happens to the toxicant or in what it does to the organism. It will be obvious that the second approach must necessarily range widely over practically the whole of insect biochemistry, including any essential biological process and the nature of the intra- and inter-cellular enzymes which control its rate. Even in mammals these are very imperfectly recognized and new ones are being discovered constantly. Hence it is not illogical to consider that any addition to the relatively undeveloped subject of insect biochemistry is a potentially practical contribution to the study and solution of insecticide resistance.

Numerous excellent reviews have discussed the researches on the fate of insecticides and their effects upon susceptible and resistant insects from various points of view but the question of how all or any of this work may be applied to the control of resistant strains has been largely neglected. The reason is not far to seek, for the connections are still obscure. But it may perhaps be of some value to raise a few questions about the relation of basic to applied work and to mention some needed lines of investigation on the problem of insecticide resistance.

Since Wiesmann's (1947) suggestion that houseflies in northern Sweden had become resistant to DDT through the selection of individuals having a less permeable tarsal cuticle there have been numerous attempts to account for resistance in terms of lowered entrance of the toxicant, e. g., Winteringham (1952) found that the cadelle larva is practically immune to DDT by contact because very little is absorbed, and to a lesser degree this is true of certain grasshoppers (Sternburg and Kearns, 1952). These are cases of natural tolerance occurring before exposure, but if in a population under selection there are individuals which absorb less of a toxicant it is certain that this will give them an advantage in survival and subsequent reproduction. An illustration is given by several strains of houseflies studied by Perry et al. (1955).

Numerous investigations have revealed in great detail the complicated structure of insect cuticle and the differences in various regions of the body. The importance of the waxy epicuticle has been stressed by Wigglesworth (1945) and recently by Ebeling and Wagner (1959), especially in connection with the uptake or loss of water from the body. Wiesmann (1957) found the tarsal epicuticle of DDT-resistant houseflies to contain a third more lipoid than that of suceptible flies. Despite its complexity of structure, the cuticle must share the general property of membranes that permeabilities for polar and for nonpolar molecules vary inversely. Thus a strain of insects which has become resistant because of lowered uptake of the relatively nonpolar chlorinated hydrocarbons should be treated with polar materials, i.e., one of relatively high solu-

bility in water such as nicotine or one of the more soluble organic phosphates or carbamates. It will be clear that this principle will also hold for compounds which act with reverse selection, i.e., one is more toxic to survivors from the other than to normal susceptible insects, insofar as lowered penetration is a controlling factor in the resistance.

Similarly, when resistance results from increased storage of a chemical in relatively nonsensitive tissues, e.g., DDT or dieldrin in fats, a toxicant of low fat solubility is much more likely to be effective than one of similar nature to the compound which failed. It must be understood, of course, that the principle of alternating polar and nonpolar insecticides will be of practical value only insofar as penetration of the cuticle, storage, or distribution among fatty and aqueous tissues of the body are controlling factors.

The inactivating reactions undergone by poisons and drugs in the animal body have long been a favorite subject in human toxicology and pharmacology and more recently similar interest has developed in physiologically active compounds in insects. Much was accomplished by direct chemical tests, e.g., the proof that resistant red scales convert hydrogen cyanide to thiocyanate; that arsenic exerts its toxic action by combining with sulfhydryl groups of essential enzymes and that this action is opposed by glutathione; that DDT is changed into DDE with loss of HCl in several resistant insect species; and the γ-hexachlorocyclohexane is degraded at least in part to pentachlorocyclohexene. But is was only after the powerful combination of paper chromatography and radiometric determination of labelled insecticides and their derivatives became available that this subject attained its present popularity.

Numerous studies have shown that in general insects degrade toxicants similarly to mammals, i.e., by forming more water-soluble derivatives through oxidation, hydrolysis, or formation of salts, esters or other complexes with body constituents thus making it easier for the excretory system to pass them from the body. Thus in the German roach, DDT is converted to a number of substances of increasing polarity among which are the hydroxy compound Kelthane[1], and dichlorobenzophenone (Hoskins and Witt, 1958). Similarly the American roach forms a complex apparently with a phenolic body constituent (Terriere and Schonbrod, 1955) and others as yet unidentified. The fruitfly, *Drosophila melanogaster*, forms Kelthane in the larval stage (Tsukamoto, 1959) and also in the adult[1]. The same principle is illustrated by the series of increasingly polar derivatives which are formed in insects and in plants from the sulfur-containing organic phosphates such as demeton (Metcalf et al., 1954). The housefly appears to be unique in that the primary reaction which largely controls suceptibility to DDT is the enzymatic loss of HCl to form dichlorodiphenyl ethylene (DDE) which behaves chromatographically as a less polar substance than DDT. Later processes, however, form the characteristically more polar and water-soluble type of derivatives as in the roach. Certain mosquitoes combine the two processes forming both DDE and polar derivatives immediately upon exposure to DDT (Hoskins, et al., 1958).

Such studies have added considerable to the available information upon normal and what may be called abnormal insect biochemistry. Special mention should be made of the classic work on the enzyme which brings about the dehydrochlorination of DDT (Sternburg et al., 1954; Lipke and Kearns, 1959; Lipke and Kearns, 1960). This enzyme, called DDT-ase for convenience, may be inhibited by a number of DDT analogs and presumably by such unrelated compounds as piperonyl cyclonene which reduce the formation of DDE in vivo in flies (Perry and Hoskins, 1951; Yasutomi,

[1] Unpublished results from the Laboratory of Insect Toxicology, Department of Entomology, University of California, Berkeley, California.

1954), but despite very extensive tests no synergist has yet been found which used with DDT brings the resistant insect back to the fully susceptible level. And under exposure to both, the effectiveness of the combination soon decreases, presumably due to selection of individuals which contain still larger amounts of the enzyme (Moorefield and Kearns, 1955).

This is a remarkable matter for two reasons. First, the failure of synergists to undo completely the acquired resistance to DDT means that no compound used to date can combine with the DDT-ase irreversibly enough to prevent its action to some degree upon DDT, which must then have such an optimum affinity for DDT-ase that in the presence of more numerous competitors (the synergist molecules) it can gain access to the enzyme und undergo the usual rapid dehydrochlorination process and separation of the reaction products. This is a much closer adjustment of enzyme to substrate than usually prevails. And it is the more surprising because the ancestral individuals in whom this enzyme first developed had never been in contact with DDT nor anything remotely resembling it.

Secondly, if the production of DDT-ase is due to a preeexisting gene having this as one of its functions there should be a limit to the amount that can be present in the homozygous resistant individual and at some level of the DDT plus synergist control should once more be possible unless the amounts needed are so great that adequate penetration is not possible. It would seem that restoration of the former effectiveness of DDT is contingent upon the discovery of a more effective synergist. In this connection Lipke and Kearns (1960), noting that certain analogs of DDT still have synergistic action after being acted upon by DDT-ase, have made the suggestion that search should be made for compounds that show this action, i.e., inhibition of the enzyme by the reaction product. The original compound need not be synergistic at all. This is somewhat similar to the situation when a comparatively nontoxic substance is activated in the body to a very toxic one, e.g., parathion to paraoxon and heptachlor to its epoxide. Certainly the discovery of a really effective synergist for DDT would have such worldwide consequences in the control of insects of agricultural, household and medical importance that no approach to the problem should be neglected.

The choice of an alternate insecticide when resistance has rendered the one in use no longer satisfactory need not be made entirely by chance. If, for instance, the chemical is subject to inactivation by oxidation and a strain having this ability to high degree has been selected, it is logical to shift to a material which is not subject to oxidation by the same mechanism. Or if failure is due to hydrolysis, a new insecticide which cannot be degraded in this manner may be selected. Herein lies the justification for detailed study of the metabolism of insecticides in susceptible and resistant insects, for a logical choice of an alternate material cannot be made unless the true cause for the failure is known. It is not sufficient to determine that several metabolites are formed in a treated insect nor even to identify each, for in any sequence of products, the rate of formation of one is the determining factor. It also may happen that interference with any step up to the last will quickly stop the whole process. Hence a logical extension of the work on metabolism of DDT in mosquitoes or in the roach is the determination of the order in which metabolites are formed and the kind of reaction concerned in the production of each. Perhaps the proper agent to block one or more of the steps can then be selected.

Despite all the work on the fate of DDT in house flies and on the properties of DDT-ase, the precise steps by which the change occurs are not known. Since the hydrogen atom on the carbon between the two benzene rings is reactive chemically, an attractive theory is that removal of this H is the key process, a Cl atom on the other carbon then leaving to preserve the electroneutrality of the resulting structure, i.e.,

DDE. This function of DDT-ase as a dehydrogenase would go far toward accounting for its presence in ancestral insects necessarily unexposed to such a chemical. It is interesting that Lipke and Kearns (1960) found the ratio of LD 50's of DDT and DDD (the dichloro analog of DDT) to be exactly the reciprocal of the relative initial rates of dehydrohalogenation of the two compounds, i. e., the magnitude of the toxic doses seems to be determined by the rates of degradation. But with compounds less closely related to DDT, e.g., the p-methoxy analog, this relation does not hold. If DDT's fundamental behavior is attachment to and inactivation of an essential enzyme or attachment to a membrane whose permeability is thereby altered, it would seem that the lability of the hydrogen on carbon number one would be of great importance. But in the absence of information on the action of DDT, such speculations are of little value.

Attempts to explain the toxic action of DDT in terms of its effects upon essential biological functions have been concerned largely with changes in the activity of certain enzymes in poisoned insects. While effects have been observed, they are small and not proportional to the toxicity. Thus DDT will inhibit cytochrome oxidase in homogenates of the mealworm and of the American roach (Ludwig et al., 1955) and succinic oxidase (Ludwig and Barsa, 1957) in eggs of the mealworm. Oxidation of intermediates of the Krebs cycle is inhibited by DDT with preparations from the housefly (Sacklin et al., 1955). Decreases in the total amino acid content of the hemolymph of flies (Reiff, 1956) and of proline in that of *Periplaneta* (Corrigan and Kearns, 1958) have been reported. Micks et al. (1960) recently reported a doubling of the alanine concentration in DDT-resistant *Aedes aegypti* larvae some four hours after exposure, but a susceptible strain showed the same change at a lower level. All such effects probably result from a change in enzyme activity. Such work can have only remote relation to the problem of overcoming resistance for it is impossible in general to alter at will the amount of any enzyme in a living creature except to decrease it by use of an appropriate inhibitor.

Recent data on the behavior of dieldrin in houseflies has led to a change in viewpoint regarding the basis of resistance to this compound and of the other members of the group. Part of an absorbed dose is changed to polar material and hence differences in this process were thought at first to account for resistance. But careful measurements using S^{32}-labelled dieldrin analog and C^{14}-labelled aldrin and dieldrin have shown no difference between susceptible and resistant strains in absorption, metabolism or excretion. The same situation prevails with γ-hexachlorocyclohexane (Bridges and Cox, 1959; Bridges, 1960). Hence some more subtle basis must be sought for the high resistance that can develop in this and other species.

Great interest followed the first claims that certain chemicals were more effective against DDT-resistant flies or mosquitoes than against the normal susceptible strains since by alternate use it would be possible to maintain a sort of oscillating balance in susceptibility. Unfortunately, to date the idea has not proved of practical value. From the biochemical point of view, reverse selection is possible only under quite restricted conditions. The individuals which survive exposure to a compound necessarily have some characteristic in exaggerated form, e.g., a less permeable cuticle or midgut lining, a detoxifying enzyme, extra fatty tissue for storage, a more efficient excretory mechanism, etc. In general, there is no reason why individuals so endowed should be more susceptible to a second chemical; in fact, the vigor tolerance usually associated with the specific resistance will tend to make them less susceptible to any adverse condition. Certainly a second substance having similar polarity and chemical properties cannot exert reverse selection. On the contrary, a compound of markedly different characteristics may have its usual distribution among body tissues altered

in the direction that will increase its usual toxic action. If a process which detoxifies the first insecticide also affects the second to form a more toxic metabolite, the ideal case of inverse selection will result.

The similarity of conditions under which HCl is lost from DDT and from the organic phosphate Dipterex in vitro under mildly alkaline conditions has led to speculation whether the same process occurs with both compounds within insects. Though the dehydrochlorination of Dipterex was denied at first by some workers, Metcalf et al. (1959) have obtained clear proof by chromatographic and radiometric methods that DDVP is formed from Dipterex in considerable amounts in houseflies. The important point in connection with reverse selection is whether this proceeds more rapidly and (or) to a greater extent in DDT-resistant flies. This matter should be studied with this and all other compounds which may be activated by the dehydrohalogenation reaction.

The foregoing discussion has attempted to emphasize that biochemical and physiological work with insects offers long range dividends in the control of insect pests but in the present state of development few applications can be made to the problem of insecticide resistance as it now exists. As the present relatively fragmentary data on insect life processes are enlarged to form a coordinated whole, the various processes that lead to resistance will be seen as illustrations of general principles. When such a time arrives the chemical control of insect pests will become transformed from an empirical art to an applied science.

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ORGANOPHOSPHORUS RESISTANCE IN THE HOUSEFLY

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In contrast to resistance to the chlorinated hydrocarbons, which is dependent on a number of unrelated physiological processes and on a number of genes, organophosphate resistance in the housefly seems to be brought about mainly by one kind of defence mechanism depending on the mutants of one single gene.

The first indication that this is so was obtained by the finding that in homogenates of all organophosphate resistant strains a very low ali-esterase activity was present, whereas a high activity was found in homogenates of other strains, either susceptible or resistant to chlorinated hydrocarbons (van Asperen and Oppenoorth, 1959). Until now this has been found to be true for seven strains either resistant to malathion or to parathion-diazinon and for six phosphate susceptible strains.

By appropriate crosses it could be shown that the difference in esterase activity is brought about by one mutant gene in each strain that is also responsible for resistance (Oppenoorth, 1959, Nguy and Busvine, 1960). The mutant genes for low esterase activity that are present in different strains are not identical, since they cause different types and levels of resistance: they turned out to be alleles of the same gene (Oppenoorth and van Asperen, 1960).

In homogenates of the resistant strains enzymes have been found (one in each strain) that degrade the oxygen analogues of the organophosphorus insecticides to which the strains are resistant (van Asperen and Oppenoorth, 1960, Oppenoorth and van Asperen, 1960). Such an enzyme is also present in a specially bred strain in which the gene for low esterase activity is combined with the genome of a susceptible strain. Therefore, the presence of the breakdown-enzyme and the absence of the ali-esterase are dependent on the same gene. It is concluded that the detoxication enzymes are produced under the influence of the mutant alleles in the place of the ali-esterase, which is produced under the influence of the wild type allele.

There are at least three types of detoxication enzymes:

(a) An enzyme that can degrade paraoxon, diazoxon and some related diethyl compounds at a rate of c. 50 $\mu\mu$ moles/hr/fly. This has been found in two Danish parathion-diazinon resistant strains (D and F).

(b) An enzyme that can degrade the same toxicants at a rate of c. 150 $\mu\mu$ moles/hr/fly and can also attack some dimethyl compounds. This has been found in two parathion-diazinon resistant strains, one from Italy (strain C) and one from the United States (K = Rutger strain).

(c) An enzyme that can attack malaoxon and some other dimethyl compounds at a rate of c. 300 $\mu\mu$ moles/hr/fly, but not the diethylphosphates. This has been found in a malathion resistant strain from the United States (G = Bethesda 45 strain). Malaoxon breakdown of the same order of magnitude has been described for fly sections by March (1959).

The breakdown capacity and specificity of the enzymes mentioned generally corresponds with the resistance found in the strains (Oppenoorth and van Asperen, 1960). An anomaly was recently found in studies of strain H (= Anderson 45 strain). This strain has a malathion resistance which is somewhat lower than that of strain G, but much higher than that of strain C. Still its breakdown capacity was found to be much lower than in these two strains. It is known that the resistance in strain H is dependent on one gene (Nguy and Busvine, 1960) which must be an allele of the gene

for low esterase activity present in the other strains. Therefore, a breakdown enzyme of the type present in the other strains could certainly be expected. The fact that it is not found may be due to unfavourable conditions destroying it in the homogenates. Perhaps *in vivo* experiments could clarify this point.

The properties of the ali-esterase and the detoxication enzymes will now be compared.

1. Reaction with the oxygen analogues of the insecticides

There is little doubt that the reaction of the esterases with the organophosphates is similar to that with the carboxy esters (Dixon and Webb, 1958). It can be represented as follows:

$$\begin{aligned} E + PX &\rightleftharpoons EPX \\ EPX &\rightarrow EP + X \\ EP &\rightarrow E + P \end{aligned}$$

where E stands for the enzyme, PX for the organophosphate, with P representing the (alkyl-O)$_2$-PO-group, and X the remainder of the molecule which varies in the different toxicants.

The ali-esterase is rapidly phosphorylated in the first steps of the reaction (van Asperen and Oppenoorth 1960), and is irreversibly inhibited since the rate of the third step, the dephosphorylation, is practically nil.

The same reaction-scheme probably applies to the detoxication enzymes. Although these are not irreversibly inhibited, the rate of the third step is still low. If it is assumed that the amount of these enzymes is equal to that of the ali-esterase of the susceptible flies (c. 10^{-11} moles per fly), the turnover numbers of the detoxication enzymes described under a, b and c can be calculated to be 0.08, 0.25 and 0.50 per minute respectively. Just as in the ali-esterase, the phosphorylation reaction proceeds rapidly, and this, combined with the low turnover number, results in very low K_m values. These are less than 10^{-8} for paraoxon and diazoxon, and somewhat higher for malaoxon. This means that even at very low concentrations of the organophosphates the detoxication takes place with maximal velocity.

If the dephosphorylation reaction is the rate limiting step in the detoxication process, it follows that the rate of detoxication of compounds with the same (alkyl O)$_2$PO-group should be equal. This has actually been found for a number of diethyl- and dimethyl-compounds (for some substances for which apparently a lower affinity is present, this maximum rate is only reached at higher concentrations or not at all).

If the detoxication enzymes react with the organophosphates in a way similar to that of the ali-esterase, it can be expected that they are inhibited by phosphates which they are unable to degrade. This was found for the detoxication enzyme described under c, which can degrade malaoxon and certain other dimethyl compounds, but no diethyl analogues. It is possible by preincubation with a small amount of paraoxon to abolish the detoxication of malaoxon.

These facts indicate that the detoxication enzymes and the ali-esterase attack the same bond in the organophosphate molecule. This should be checked, of course, by identification of the reaction-products.

It is curious that no degradation at all is found in homogenates of susceptible flies, whereas *in vivo* breakdown has been reported for several insects. In the housefly Krueger and O'Brien (1959) found a number of metabolites of malathion after topical application of this insecticide. March (1959) reports breakdown in sections of susceptible flies that were incubated with malaoxon. The fact that no such degradation takes place *in vivo* is of great help in the study of the resistance mechanism, since the only reaction found in the homogenates seems to be that which is the cause of the resistance.

2. Reaction with the carboxyesters

The ali-esterase activity of resistant flies is c. 20% of that of susceptible ones for methylbutyrate, and a higher percentage for most other substrates (van Asperen and Oppenoorth 1959). This activity in the resistant strains has now been shown to be largely due to the action of other enzymes than the breakdown enzymes. Evidence was obtained, however, that the detoxication enzymes still possess a hydrolytic capacity for some substrates, among which methylbutyrate, which is only a few percent of that of the ali-esterase in the susceptible flies. These experiments will be described in detail elsewhere.

The similarity between the breakdown enzymes and the ali-esterase in their reactions both with the organophosphates and the carboxyesters strengthens the belief that the breakdown enzymes are "mutant ali-esterases".

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STUDIES ON RESISTANCE TO ORGANOPHOSPHORUS INSECTICIDES IN THE HOUSE FLY, MUSCA DOMESTICA L., AND THE MOSQUITO, CULEX TARSALIS COQ.

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During the last 2 years we have conducted several studies at the Corvallis, Oregon, laboratory comparing nonresistant and resistant strains of insects in their responses to organophosphorus and carbamate insecticides. The work has been primarily with measurements of esterase activity and insecticide metabolism. The following presentation summarizes some of our results.

Organophosphorus resistance in the house fly

Following the report of Van Asperen (1958) on the high level of aliphatic-esterase (Ali-E) inhibition that occurred in house flies (*Musca domestica* L.) poisoned with DDVP, we undertook a study of the comparative importance of cholinesterase (ChE) and Ali-E inhibition in the toxic action of organophosphorus insecticides. Independently

of Van Asperen & Oppenoorth (1959), but some months later, we observed the occurrence of low levels of Ali-E activity in strains of flies resistant to organophosphates. In a colony selected by exposure to parathion and about five times resistant to it, Ali-E activity was 40% of that of a nonresistant colony. In a strain 50 times resistant to malathion, Ali-E activity was about 60% of that of the same nonresistant colony. We also found that Ali-E activity in both nonresistant and resistant strains was five times more susceptible to inhibition by parathion and paraoxon than ChE under *in vitro* conditions, whereas with malathion, ChE activity was twice as susceptible to inhibition as Ali-E. This work has been published recently (Bigley & Plapp 1960) and need not be discussed further.

Studies were then undertaken to compare the pattern of inhibition of the two esterases *in vivo* in flies treated with parathion or malathion. In all experiments, measurements of enzyme activity were made by the colorimetric method of Hestrin (1949). Acetylcholine and methyl n-butyrate were used as substrates for ChE and Ali-E, respectively. ChE activity was determined in breis prepared from the heads of treated flies and Ali-E activity was determined in trunk (thorax plus abdomen) breis of the same flies. The technique in which samples of treated insects were homogenized in substrate to protect the enzymes from further inhibition during the course of sample preparation was used for all analyses.

The results of a typical experiment in which nonresistant flies were treated with an LD-50 of parathion may be summarized as follows: Ali-E activity was inhibited maximally (to about 50% of normal) within 15 minutes after treatment. By the time the flies showed symptoms of intoxication (about 2 hours later) the inhibition was declining, and by knockdown (4 hours after treatment) Ali-E activity had recovered to 92% of normal. Twenty-four hours after treatment, activity of the enzyme had recovered to the pretreatment level. The inhibition of ChE activity was closely correlated with the symptoms of poisoning. For the first half hour, little inhibition occurred, but a progressive decline followed so that ChE activity was 70% of normal when the flies first showed symptoms of hyperactivity. At 4 hours after treatment, maximum inhibition to 31% of the pretreatment level was observed. At 24 hours, ChE levels in surviving flies had recovered to about 50% of the pretreatment level.

Very similar results were obtained in studies with a parathion-resistant strain of house flies treated at their LD-50. Here, also, maximum inhibition of Ali-E activity occurred almost immediately after treatment, whereas the pattern of ChE inhibition was closely correlated with the symptoms of poisoning. The same pattern was observed in flies treated with malathion. Here, although *in vitro* experiments had shown ChE was more susceptible to inhibition than Ali-E, maximum inhibition of Ali-E to about 50% of normal occurred within a few minutes after treatment. As with parathion, the inhibition of ChE was delayed and maximum inhibition occurred at knockdown. Ali-E activity was normal 24 hours after treatment, as with parathion, whereas little recovery of ChE occurred in flies surviving treatment with malathion.

The results of these experiments may be summarized as follows: The pattern of ChE inhibition was closely correlated with the symptoms of poisoning following treatment with both parathion and malathion, while the pattern of Ali-E inhibition could not be directly correlated *in vivo* with the toxic action of the insecticides. In all experiments in which we produced any toxic effects Ali-E activity was inhibited; nevertheless, our results indicate that the inhibition was not closely correlated with the symptoms of poisoning and was not the direct cause of death.

In other experiments we determined the levels of ChE and Ali-E activity and the susceptibility to parathion at all stages in the life cycle of a parathion-resistant and a nonresistant strain of flies. We found that levels of ChE activity were nearly identical

at all stages in the two colonies, whereas levels of Ali-E were lower in larvae, pupae, and adults of the resistant strain. Larvae and pupae of the parathion-resistant strain were, like the adults, less susceptible to the insecticide than the comparable stages of the nonresistant strain.

Malathion resistance in *Culex tarsalis*

Resistance to malathion in the important pest species of mosquito, *Culex tarsalis* Coq., vector of western equine encephalitis in the United States, was first reported from California by Gjullin & Isaak (1957). At the present time a colony is maintained in our laboratory about 50 times more resistant to malathion than a corresponding nonresistant strain also maintained.

In studying malathion resistance in this species, we determined the range of cross-resistance to other insecticides. We found that the resistant colony was about 7 times resistant to both the oxygen analog of malathion (mala-oxon), and the diethyl homolog of malathion when compared with the nonresistant colony. We observed no cross-resistance to any others of a large series of phosphate insecticides. The materials tested included both diethyl and dimethyl phosphates and phosphorothioates, and several compounds in which detoxication by formation of carboxylic acid derivatives, as with malathion, is either known or possible. Of particular interest was the fact that cross-resistance did not extend to acethion (0,0 diethyl S-carboethoxymethyl phosphorodithioate). As described by O'Brien et al. in 1958 acethion exhibits a pattern of selective toxicity similar to malathion.

In work with nonphosphorus insecticides we found that resistance to DDT and dieldrin was present only to a factor of 2—3 times in the malathion-resistant colony and there was no cross-resistance at all to the carbamate insecticide Sevin (1-naphthyl methylcarbamate). These results may be contrasted with the typical pattern found in the house fly in which resistance to one phosphate usually involves cross-resistance to many related materials and almost always very high levels of resistance to materials such as DDT and dieldrin.

By means of radioactive malathion, the metabolism of the insecticide *in vivo* by larvae of the resistant and a nonresistant strain was compared. Our tests showed that the rate of breakdown of malathion was almost identical by larvae of both colonies. Furthermore, the metabolism was the same; both colonies detoxified malathion largely by hydrolysis of the carboethoxy bond to give a carboxylic-acid derivative. We obtained similar results with acethion; again we observed no difference in the rate of metabolism between the colonies, and the carboxylic acid derivative was the major breakdown product.

Our studies on esterase activity in *tarsalis* have shown that, as with flies, there were no differences in either levels of or susceptibility to inhibition of ChE activity between the colonies. There was no Ali-E activity to methyl butyrate in either strain of mosquito. Ali-E activity was demonstrated in experiments in which tributyrin was the substrate. However, no differences in levels of Ali-E activity between the two colonies were observed and the activity present also differed in that it was completely insensitive to inhibition by malathion and parathion at levels of 1×10^{-4}, or lower.

Thus, a pattern of resistance occurred that was very different from the one in flies. Cross-resistance to other insecticides was limited. The Ali-E, which declines in resistant flies, was not present in the mosquitoes. Our studies failed to elucidate the nature of the mechanism responsible for resistance to malathion, either in terms of esterase activity or in a difference in the rate of metabolism. It is interesting to note that the pattern is very different from that reported for *Aedes aegypti* by Brown & Abedi (1960), in which resistance to malathion gave a high degree of cross-resistance to DDT.

Effect of carbamates on ChE and Ali-E activity in house flies

More recently we have performed some experiments on the inhibition of esterases in house flies by carbamate insecticides. The inhibition of ChE in flies treated with LD-50's of Sevin and Isolan (5-[1-isopropyl-3-methylpyrazolyl] dimethylcarbamate) was determined. We noted good correlation between the pattern of inhibition and the observed symptoms of poisoning. ChE inhibition to 30% of normal or less occurred shortly after treatment. As the flies recovered, the inhibition declined so that survivors showed normal ChE activity within 24 hours.

We also determined the effects of Sevin and Isolan on Ali-E activity in house flies. *in vitro* experiments showed that house fly Ali-E activity was very susceptible to inhibition by both compounds. The amount required to inhibit Ali-E activity was about 10 times that required for ChE, or about 5×10^{-6} molar. When flies were treated with LD-50's of either Isolan or Sevin *in vivo*, we found the pattern of inhibition of Ali-E very similar to that with ChE. This may be contrasted with parathion and malathion in which the patterns of inhibition of ChE and Ali-E are markedly different.

In summary, recent experimental work at our laboratory has been concerned largely with the relationship between low Ali-E activity and resistance to insecticides. We have found that the pattern of Ali-E inhibition, *in vivo*, cannot be directly correlated with the toxic action of organophosphorus compounds to the house fly. In the mosquito, *Culex tarsalis*, we found no relationship between Ali-E and malathion resistance. Recent work with the carbamate insecticides, Sevin and Isolan, has demonstrated that Ali-E, like ChE, is susceptible to inhibition by these compounds.

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THE GENETICS OF RESISTANCE

R. MILANI

A number of surveys on the genetics of resistance have been published in the recent years, so this presentation will cover only the most recent papers, which, beside broadening the available case-history, add new significant information.

Male limited (holandric) inheritance of resistance

An unusual model of inheritance of resistance has been found by R. K. Kerr (1960) in a laboratory DDT-resistant strain of house fly. The strain derives from the Canberra laboratory colony bred since 1939 in isolation from insecticides. The strain has an history of selection for early maturation, followed by DDT selection.

Before both selections, the strain contained a small proportion of DDT-resistant flies of both sexes. Selection for early maturation caused the disappearence of resistant females by the sixth generation, and an increase in the proportion of resistant males up to about 75 per cent after a further 68 generations.

At this stage, a single selection with a topically applied dose of 32.0 μgr. DDT/gr. body weight allowed complete separation of resistant males and the establishment of a true breeding strain with DDT-resistance limited to the male flies.

In the selected strain resistance is manifested only in males and is transmitted in crosses only by males to the male progeny. Kerr points out that resistance behaves as if it were caused by a factor located on the Y Cr. or by a balance mechanism like sex.

A similar type of inheritance is known for morphological characters.

With flies of american origin (Milani 1958; Sullivan 1958; Milani & Franco 1959) exceptional strains have repeatedly been established with females homozygous for recessive mutants of the II° Chr., like *bwb*, *green eyes*, and *white eyes* and males phenotypically normal but genetically heterozygous. The locus *bwb*, the only one of which I have direct experience normally behaves as autosomal. The simplest interpretation is that a translocation involving II° and Y Chromosomes causes the holandric (male limited) type of inheritance; cytological support of this hypotesis is at present missing.

It should be pointed out that the holandric type of inheritance is expected even if the translocation does not actually include the marked loci, because in the heterozygous condition the two chromosomes with the translocation go always to the same pole, and their normal homologous to the other, when reduction occurs, during meiosis.

The II° Chromosome carries at least two independent genes for resistance, *kdr* (Latina) and *kdr-o* (Orlando), which suggest a closer connection with the case of Kerr (Milani e Travaglino, 1957; Milani & Franco, 1959).

If a recessive factor for resistance follows the Y Chromosome it could not reach the homozygous condition and cause full resistance unless translocations or crossingover change the linkage relation; we have had evidence of such a case in crosses involving a Orlando R (= Lab. 1) male (Milani, 1959a).

It is obvious that the presence of chromosomal polymorphism of this type can cause the formation of strains with susceptible females and resistant males, or the reverse, according to the chromosomal localization of the gene(s) for resistance. In both cases, homozygous strains showing resistance in both sexes can be obtained only when crossing-over breakes the binding[1] with the Y Chr.; this is a very rare event for the marker *bwb*.

These are special cases of mendelian inheritance, but they can (a) introduce intriguing complications in crossing experiments; (b) hinder the response to selection, which will become sudden and "explosive" when crossingover releases factors othervise binded to the Y.

Coexistence of more than one factor causing resistance of similar type

Evidences for the presence of more than one factor for DDT-resistance have recently been found in two strains of housefly. DDT-resistance of Lindquist's strain Lab. 1 (Orlando-R in papers by Milani and Travaglino, 1959; and Milani and Franco, 1959) is mainly caused by a recessive factor, *kdr-o* of Chr. II°; some flies however, carry a modifier of dominance, causing full resistance to the heterozygous.

[1] The word "binding" is used to include true linkage and no-recombination between mutually translocated chromosomes.

A field resistant strain (Dwight, Ontario) carries on the II° Chr. at least one, possibly two other independent factors, each capable of causing differently delayed knock-down and resistance (Franco, 1959; unpublished data). In the latter case more than one factor has been recognized in a single strain, but-under the testing condition, which are somewhat comparable to field treatment-their coexistence is not required for assuring protection.

Both the Lab. 1 and Dwight strains have shown prompt response to selection for early or delayed knock-down, but in the selected strains some "leaking" of resistant or of susceptible flies showed that homogeneity was difficult to reach.

The presence of dominance modifiers and of mimic genes (i.e. different genes providing similar effects) provide sufficient explanation of the tendency to revert toward susceptibility, because both conditions foster the persistence of genes for susceptibility among flies of similar (high) resistance.

Pleiotropy; suggestions and evidences

Shanahan has found that dieldrin resistance in *Lucilia cuprina* gives F_2 segregations in agreement with expectation for a genetically simple caracter; moreover, homozygous resistant flies can be recovered in agreement with the hypotesis of monofactoriality after a series of backcrosses to the susceptible strain. Dieldrin has been the selecting factor, but resistance extends to aldrin and BHC. Aldrin and BHC resistance are also inherited as genetically simple properties; the heterozygous can be fully discriminated from either homozygous using aldrin or dieldrin, but partly overlap with homozygous susceptibles when tested with BHC (Shanahan, 1959).

The further development of this research still in progress will clarify if a single gene action or different actions of the same gene or different genes are at play and will contribute to the understanding of the genetic basis of cross resistance; very preliminary information suggest the first possibility as the most likely.

The genetics of dieldrin resistance in *Lucilia cuprina* is fully in accordance with the finding of Davidson (1958) on resistance of *A. gambiae* to the same toxicant. The parallelism extends to (a) the type of dominance which is intermediate in both cases, (b) the close agreement of observed segregations with expectation on a simple mendelian basis, (c) the possibility of recovering full resistance after a series of backcrosses to the susceptible, (d) the indication of a common genetical basis for dieldrin and BHC resistance. The only (slight) point of disagreement is that, contrary to *A. gambiae*, resistant *Lucilia cuprina*, shows tendency to reversion toward susceptibility after some six generations on release of insecticide pressure.

Drosophila melanogaster provides examples (a) of the coexistence in the same strains of more than one locus causing resistance and (b) of a different specificity for the action of each single gene. Genes on loci II, 64.5 and III, 50 cause different degrees of DDT, BHC, parathion, phenilthiourea resistance; only genes of locus III, 50 control nicotin resistance; beside that, the two loci affect in opposite ways the normal tolerance to phenilurea (PU), which is decreased by the resistance allele(s) of locus II, 64.5 and increased by those of locus III, 50 (Kikkawa, 1958; Tsukamoto et al., 1957; Ogita, 1958).

That means that from the same strain it is possible to isolate two resistance factors showing in one a positive and in the other a negative correlation between resistance to PU and resistance to other chemicals.

Multiple allelism and resistance to related chemicals

Nguy and Busvine (1960) have been able to show that two genes inherited in opposition cause resistance one to parathion the other to malation; the genes derive, respectively from italian and american fly populations.

No crossingover has been observed between them, suggesting allelism or close linkage; Oppenoorth (1959) has proved that this is a case of allelism and that a third allele exists which cause a different degree of parathion-diazinon resistance.

Physiogenetics of resistance

The physiogenetics of resistance has received a good start from the work of Lovell and Kearns (1956, 1959) on the inheritance of dechlorinating activity and of Oppenoorth (1959) on the inheritance of low ali-esterase activity, as found in organophosphorous resistant strains.

Dechlorinating activity can be measured on individual flies and is very low, intermediate or high according to the genetic constitution of the flies, namely homozygous susceptible, heterozygous, homozygous resistant. In crosses it follows exactly the same pattern of inheritance given by the response to toxicological test.

Low ali-esterase activity, as found in OP-resistant strains, is inherited as a monofactorial in coincidence with resistance. In different strains three alleles have been recognized having equal effect on ali-esterase activity but differing widely in the specificity and degree of break-down capacities and of the resistance which they confer (Oppenoorth, 1959). Moreover from a diazinon resistant strain a resistance factor has been found which is independent of low ali-esterase activity. As with the above mentioned flies from Dwight and the drosophilas, different factors have been found coexisting in the same strain, capable of independently protecting the insects from intoxication; the Oppenoorth data show that they may have very different biochemical properties.

Summing up, in the last few years the genetical work has contributed to the problem of resistance by showing that:

(a) factors for resistance in the house-fly can follow unusual patterns of mendelian inheritance (holandric);

(b) more than one factor can coexist in the same strain, causing similar forms of resistance;

(c) the dominance relations between alleles of loci involved in resistance can be changed by modifying genes;

(d) a factor causing cross-resistance to two related insecticides can behave as an incompletely recessive with one and as intermediate dominant with the other;

(e) resistance to one insecticide can be coincident with exceptional susceptibility to other toxicants; at least in one case (phenilurea, PU, in *Drosophila*) negative correlation seems to be an instance of pleiotropy, limited to one of two genes having partially overlapping ranges of action;

(f) different genes—possibly alleles, not having provided crossovers—control resistance to parathion or to malation, of the same chemical group;

(g) the enzymatic properties distinctive of resistant strains are inherited as genetically simple characters, like resistance and jointly with resistance.

General considerations

The development of resistance can be visualized either as a process of concentration of factors of individually little effect, but cumulative in action, or as an increase in the frequency of insects endowed with genetically simple defence mechanisms capable of providing full protection.

The development of resistance in the field has often been described as a slow process; the experiments on population dynamics with drosophilas unanimously point to the

building up of complex (integrated) polygenic systems in development of resistance, and so these two sources of information converge in suggesting a complex genetic basis of resistance.

On the other hand, fully resistant insects sharply distinct from susceptible, have been found both in field untreated populations and in long established laboratory colonies with no history of treatment; instances of explosive out-breaks of field resistance are on records; beside that, most of the work on the static aspects of resistance—namely toxicological, biochemical, physiological and genetical (on inheritance)—present susceptible and resistant insects as endowed of alternative properties rather than as extreme variants of a continous range.

Work on the inheritance of house fly resistance has shown that simple inheritance can be of holandric type. This is an isolated case, but provides a very clear model for genetic situations in which even a single gene is strongly prevented from involving all the individuals in a population. In this case selection acts on one sex only, but would cease to do so when and if inter—or intra—chromosomal rearrangements should break the sexlimitation.

We have seen also that more than one factor for resistance, individually recognizable but not necessarily additive, have repeatedly been found in different species, and for different types of resistance.

Analytical work would allow in both cases recognition and isolation of specific factors for resistance; but work on the development of resistance would undoubtedly indicate that response to selection is a process dynamically complex. These considerations should reconcile some divergence of opinion about the genetic basis of resistance deriving from a too rigid use of the terms monofactorial, multifactorial and polygenic. These terms are not mutually exclusive.

A factor with measurable effect can be part of a genetically complex system. Moreover the same genetic unit can have different levels of action, one (or some) causing a sharp deviation from normality, and others contributing but little to quantitative traits (say e.g. a mutant color and a lowering of fertility or of developmental rate); the major effect may be differently detectable under different conditions. Applying this to examples from resistance, a gene R causing high resistance to insecticide A, may contribute some resistance to B, when other factors are present; it would be recognized as a simple mendelian factor or as a polygene according to whether A or B are used for tests on inheritance; selection with B, acting on R only, would have little direct effect, but if R should ensure a small but consistent protection against B it would be selected for and cause an enormous rise in resistance to A.

This could be proposed as a working hypothesis for the results of the selection experiments of Meltzer (1956) with S-17. This insecticide "scarcely rised tolerance to itself, but increased organophosphorous resistance by 6 times, the DDT resistance by 15 times and Dieldrin resistance by several thousand times" (Brown, 1959, pag. 24).

Resistance has developed both under field and under laboratory conditions; evidence for both simple and complex inheritance of resistance have been produced.

It seems worth considering if any relation exist between condition under which resistance has developed and its genetical basis; the 14 species and more than 50 independent strains studied, provide a reasonable amount of information, even if the grouping of experiments according to two criteria, namely, origin of resistance and type of its inheritance, is not always easy. Not all authors have reported full informations on the origin of the strains used; the interpretation of the results is sometimes open to criticism.

The grouping of the experiments counted here is based on the interpretations of inheritance given or suggested by the various authors.

Agreeing results based on the same strain—or on strains of common origin—have been counted as a single piece of information; data from the same strain but in disagreement have been counted as independent sets of information. This has little bearing on the over-all picture, because contrasting interpretations have been published only for one strain of housefly, and one of *Drosophila melanogaster* (Torre in Pietra and Hikone respectively; the contrast is perhaps merely formal according to Milani, 1956—1958).

According to this presentation, polygenic inheritance has been claimed only for 8 out of 31 strains of field developed resistance and in 10 out of 16 laboratory strains ($\chi^2 = 6.0$; $P < 0.02$). The difference in proportion would become even more striking if only a critical choice of published evidences were taken into consideration. Moreover only three out of 14 species, namely housefly, german roach and drosophila have provided suggestion of polifactoriality.

The fact that, whether we consider strains or species, field developed resistance should be most frequently caused by simple genetical mechanisms might appear against expectation, if expectation is to be based on the evidence of:

(a) selection experiments on domestic animals and cultivated plants;
(b) population genetics.

Improvement of production and adaptation to a variously changing environment are slow delicate and complex processess even when directed by a simple selective stimulus, as in some experimental conditions; the results can be quite spectacular, but the difference between selected and unselected strains never compares with the difference between most resistant and susceptible strains.

I feel that much caution should be adopted before assuming that knowledge provided by the study of adaptive responses directed by the experimenter or reached under a natural slowly and variously changing environment should necessarily be fully valid for explaining the survival to genocide.

Analogies more directly relevant to the point at issue can easily be found among the numerous single gene differences in enzymatic activities which form the body of biochemical genetics (Wagner, R. P. & Mitchell, H. K., 1955) and among the numerous forms of resistance to disease or to parasites or to exceptionally harsh conditions controlled by simple genetical mechanism.

* * *

One aspect of the resistance problem which has received much attention in recent times is that of negatively correlated insecticides.

Negative correlation between DDT-resistance caused by locus II, 64.5 and PU in *Drosophila* has been mentioned; opinions are in desagreement about negative correlation between bromoacetate and DDT in housefly (Asher, 1957; Keiding, 1958).

The genetic basis of negative correlation can be manifold:

(a) different genes causing resistance to A and high susceptibility to B, are present in the same strain either because of close linkage or as a consequence of genetic drift;
(b) the same genes control more than one property, and resistance to A and susceptibility to B are distinguishable actions of the same gene with no obvious mutual dependance.
(c) the defence mechanisms to A is or becomes an hindrance in presence of B.

Negative correlation of type (a) is obviously due to chance, and cannot have long lasting or vide-spread practical utility; type (b) and more so type (c) may be expected to be ever lasting being due to intrinsic properties of the responsible genes. However,

pleiotropic effects can be independently altered by modifying genes; moreover, a defence mechanism to B quite independent from A, can be selected for and overcome the negative correlation.

I personally visualize negative correlation specially of type (c) as an effective and, possibly, long-lasting remedy; not as an ever lasting one. After all, synergists have already provided a clue to this.

LITERATURE

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DDT-RESISTANCE-INDUCED ENHANCED SUSCEPTIBILITY TOWARDS CETYL FLUOROACETATE (CFA) AND CETYL FLUORIDE (CF)—A PRELIMINARY REPORT[1]

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ABSTRACT

"Resistance-induced enhanced susceptibility", a new attempt to stave off insecticide resistance (Ascher and Kocher 1954) has been investigated in several laboratories during the last 6 years. Its present state has been recently reviewed (Ascher 1960).

In continuation of work on CBA, cetyl bromoacetate (Ascher 1957, 1958), which was found to be negatively correlated to resistance in several housefly strains on continuous contact of adult females (cf. however Keiding 1958, Bettini, Boccacci and Natalizi 1958), a group of further cetyl esters[2] was investigated. Of these, cetyl fluoride (CF) and cetyl fluoroacetate (CFA) were more active towards *larvae* of DDT-resistant houseflies. Larvae of the DDT-resistant housefly strains K1 and TP were affected more strongly by these two compounds than larvae of the normal reference strains Sv and S-Rome (2nd stage larvae added to media containing different concentrations of the toxicants; results read from hatch of adults). No negative correlation to resistance was found in the chlordane-resistant strain R-Sard.

In a number of DDT-resistant strains of *Anopheles atroparvus*, namely RL, RLAF and RAFM (Mosna, Palmieri, Ascher, Rivosecchi and Neri 1959), all developed from the same reference strain Sens.-Roma, clear cut negative correlation to resistance was found with CF in larval state assays. Another normal reference strain of *A. atroparvus*, Sens.-Hamburg, was included in these studies. LC_{50}'s (expressed in ppm) were as follows:

a) towards DDT — RL = 1.1 > RLAF = 0.31 > RLAF = 0.072 > Sens.-Roma = 0.029 > Sens.-Hamburg = 0.021 ppm.

b) towards CF — Sens.-Hamburg = 21 > Sens.-Roma = 18 > RAFM = 16 > RLAF = 14 > RL = 12 ppm.

There was no resistance-induced enhanced susceptibility to CF, CFA, cetyl chloroacetate, cetyl cyanide, cetyl thiocyanate and cetyl bromide in the *adult* houseflies and anophelines[3].

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[1] Study executed under a WHO grant in 1958/59 as research scholar at the Istituto Superiore di Sanità, Rome, Italy. Opinions and assertions contained therein are the personal ones of the author and are not to be construed as reflecting the views of the Istituto Superiore di Sanità and the World Health Organization at large.

[2] The compounds prepared for this study were synthesized by Prof. E. D. Bergmann, Tel-Aviv, Israel.

[3] A full account of the study will be given elsewhere.

SYMPOSIUM XII

EPHEMEROPTERA

EINLEITUNG

Leiterin: Prof. Dr. GERTRUD PLESKOT

Das Symposium war von 20 Teilnehmern aus Großbritannien, Rußland, Amerika, Tschechoslowakei, Deutschland, Schweden, Ägypten, Brasilien und Österreich besucht. Die angesagten Teilnehmer aus Bulgarien und Jugoslawien waren nicht gekommen. Das Fehlen von Teilnehmern aus Frankreich, Schweiz, Italien, Belgien, Rumänien und Polen wurde sehr bedauert.

Am ersten Nachmittag wurden die im folgenden veröffentlichten Referate gehalten. Auf Wunsch der Teilnehmer wurde ein zweiter Nachmittag für eine allgemeine Diskussion aktueller Probleme der Ephemeropterenforschung vorgesehen.

Bei dieser Diskussion zeigte sich, daß die allgemeine Sorge der taxonomischen Situation gilt. Familien und Gattungen sind zwar fast durchwegs klar, aber ein sehr hoher Prozentsatz der Arten bedarf noch der Bearbeitung.

Für die Klassifikation der Imagines wurde eine genaue Beschreibung der Genitalorgane nach mikroskopischen Präparaten von flüssig konserviertem Material und die möglichste Vermeidung von Färbungsmerkmalen für die Differentialdiagnose gefordert.

Da vielfach ohne Heranziehung der Larven eine Artunterscheidung nicht durchführbar ist, wurde die Wichtigkeit der Gewinnung eines Larven-Imago-Materiales durch sorgfältige Aufzucht als Basis für die taxonomische Arbeit betont. Erfahrungen und Methoden beim Transport lebenden Materiales und bei der Aufzucht wurden besprochen. J. Hanson (USA) wurde gebeten, seine interessanten Methoden zu publizieren; Dr. Macan nahm die Arbeit für den Druck in den Mitt. int. Verh. Limnol., die er herausgibt, in Aussicht.

Für die Bearbeitung von Fragen der Faunistik, der Verbreitung und Zoogeographie der Arten ist es in dieser Gruppe unbedingt notwendig, daß auch die Larven zur Art bestimmbar sind. Es wurde betont, wie dringend die Erarbeitung objektiv feststellbarer Merkmale an den Larven ist, wobei wieder sowohl Färbungsmerkmale wie Größenunterschiede als alleinige Trennungsmerkmale möglichst zu vermeiden wären.

Es wurde wiederholt darauf hingewiesen, daß es für die Beurteilung faunistischer Angaben angesichts der vielfach ungeklärten taxonomischen Situation eine große Hilfe bedeutet, wenn angegeben wird, nach welchem Schlüssel oder nach welcher Einzelbeschreibung die Artbestimmung erfolgt ist.

Für die faunistische und ökologische Bearbeitung der Ephemeropteren wurde neben der Bestimmbarkeit der Larven die Kenntnis des Entwicklungszyklus der Arten als notwendige Voraussetzung bezeichnet. Denn obwohl jede Art zu verschiedenen Jahreszeiten einen verschiedenen „Aspekt“ bietet, wird doch bei den meisten Freiland-

aufnahmen ein ganzjähriges Sammeln nicht möglich sein. Auch sind viele Arten nicht zu allen Jahreszeiten im gleichen Biotop zu finden.

Für die Ausarbeitung der Entwicklungszyklen aus regelmäßig entnommenen Proben stimmten die Teilnehmer darin überein, daß es sich empfiehlt, das Fortschreiten der Entwicklung nicht nach Längenmaßen (sei es nach der durchschnittlichen Größe in einer Probe oder nach Millimeter-Größenklassen) zu beurteilen, sondern nach den verschiedenen Etappen der Metamorphose, wie sie z. B. anfangs nach der Ausbildung der Kiemen und Augen, später nach den Flügelscheiden und anderen Körperproportionen unterschieden werden können. Besonders bei Gattungen mit mehreren, durch ihre Größe unterschiedenen Generationen würde das Längenmaß ein falsches Bild des Entwicklungsablaufes geben. Aber auch sonst geht das Fortschreiten der Metamorphose nicht immer mit der Längenveränderung parallel.

Man kam überein, daß es zur Verbesserung der taxonomischen Situation nötig wäre, für die schwierigsten Gattungen Revisionen nach modernen Untersuchungsmethoden und für das ganze Verbreitungsgebiet der Arten durchzuführen. Folgende Kollegen stellten sich für die Revision der europäischen Arten folgender Gattungen zur Verfügung:

T. T. Macan (Großbritannien) für *Cloeon* und *Caenis*.

I. Müller-Liebenau (Deutschland) für *Baëtis*.

V. Landa (ČSR) für *Ecdyonurus* und *Rhithrogena*.

Diese Kollegen ersuchen um Übersendung von Vergleichsmaterial.

Die Publikation einer Weltliste der Ephemeropterenarten wäre sehr wünschenswert. Es wurde beschlossen, Herrn G. F. Edmunds Jr. (USA), den Begründer und Herausgeber der wertvollen Eatonia, zu ersuchen, eine solche auszuarbeiten.

Weiters wurde beschlossen, festzustellen, wo die Typen (Holo- oder Neotypen) der europäischen Ephemeropterenarten derzeit verwahrt sind bzw. welche Typen noch existieren. Dr. Pleskot wurde ersucht, eine entsprechende Liste möglichst bald in Druck zu bringen.

Bezüglich rein nomenklatorischer Probleme stellte sich Dr. Brinck (Schweden, Mitglied der Nomenklatur-Kommission) entgegenkommenderweise zur Verfügung, für Kollegen, die sich auf diese Seite der Arbeit, die in ihrer Bedeutung für die taxonomische Ordnung in der Gruppe nicht unterschätzt werden darf, nicht einlassen wollen, die nötigen Schritte bei der Nomenklatur-Kommission zu unternehmen.

Prof. Tschernova willigte ein, eine Zusammenfassung der umfangreichen russischen Ephemeridenforschung in einer der westeuropäischen Sprachen zu schreiben, damit diese Ergebnisse auch denen zugänglich werden, die nicht russisch lesen können.

In der Diskussion war mehrfach angeregt worden, dem Symposiumbericht nationale Literaturlisten über Ephemeropteren anzufügen. Für Großbritannien, die Tschechoslowakei und Österreich ist dies geschehen. Ferner sei auf die in der Eatonia (Herausgeber G. F. Edmunds Jr., USA) laufend erscheinenden Literaturnachrichten hingewiesen und ersucht, daß alles zur Vervollständigung dieser Listen getan werden möge. Auch bezüglich der Anregung während des Symposiums, eine Liste der gegenwärtig arbeitenden Ephemeropterologen zu geben, sei auf die Adressenlisten in der Eatonia hingewiesen. Dieses Blatt stellt eine wertvolle Möglichkeit der Kommunikation während der Arbeit dar.

Zuletzt wurde beschlossen, daß dieses Symposium, das den Teilnehmern interessant, wertvoll und erfolgreich erschienen war, in weiteren Treffen fortgesetzt werden sollte. Dr. Macan wurde gebeten, ein zweites Ephemeropteren-Symposium bei der nächsten passenden Gelegenheit (voraussichtlich während des 15. Internationalen Limnologenkongresses, 1962, USA) einzuberufen.

STUDIEN ÜBER EPHEMEROPTEREN IN DER UdSSR

O. A. TSHERNOVA

Planmäßige Studien über Ephemeropteren wurden erst in den dreißiger Jahren begonnen; bis zu dieser Zeit waren für das gesamte Rußland nur 24 Arten bekannt. Gegenwärtig sind in der UdSSR schon 200 Arten nachgewiesen, von welchen 60 erstmalig beschrieben sind. Großes Material aus dem Norden des europäischen Teiles der UdSSR und aus einigen Gebieten Sibiriens und des Fernen Ostens haben zoogeographische Schlußfolgerungen für die ganze Paläarktis ermöglicht. Eine Reihe von Arten, die früher als skandinavische Endemismen angesehen wurden, sind nicht nur in den Becken der Petschora und der Flüsse des Nordurals, sondern auch in Sibirien und im Amur-Becken aufgefunden worden. Für den Norden des europäischen Teils der UdSSR wurde außerdem eine allmähliche Verminderung der Zahl der weit verbreiteten europäischen und westeuropäischen Arten in der Richtung von Südwesten nach Nordosten und auch eine Zunahme der Zahl von nördlichen Arten in derselben Richtung nachgewiesen. Der äußere Norden und das Petschora-Becken werden noch durch echte Euarkten bereichert. Die genaue Vorstellung von der Fauna der paläarktischen Region ohne die Kenntnis der Fauna des Amur-Beckens und des Fernen Ostens ist nicht möglich. Die Analyse der Fauna ist so durchzuführen, daß man in ihrem Bestande die Verbreitung und Nähe bestimmter Elemente zu den Faunen ermittelt, die mit der Paläarktis benachbart sind.

Es ist dabei nicht zu vergessen, daß bis heute keine einheitliche Meinung bezüglich der Grenzen zwischen der paläarktischen und orientalischen Region existiert. Die Frage über die Zugehörigkeit dieser oder jener Art zur paläarktischen oder orientalischen Fauna kann aber ohne die Lösung der Frage über die Grenzen dieser Gebiete nicht geklärt werden. Außerdem war unser Wissen der Eintagsfliegen des Ostgebietes nicht vollständig. Es war deswegen notwendig, bevor man mit der Analyse der Fauna des Fernen Ostens beginnen konnte, alle Angaben über die Verbreitung der Ephemeropteren in den verschiedenen zoogeographischen Gebieten zu sammeln und kritisch zu bewerten. Auf diese Weise konnte die Charakteristik der Faunen dieser Gebiete erlangt werden. Die Ephemeropteren des Fernen Ostens sind sehr eigenartig und unterscheiden sich stark von den europäischen. Die transpaläarktischen und nordeurasiatischen Arten bilden nur $^1/_5$ der gesamten Zahl der Eintagsfliegenarten des Ostens. Die übrigen $^4/_5$ der Fauna sind in Ost-Asien verbreitet. Zu ihnen gehört eine große Gruppe von thermophilen Formen, die außer dem Amur-Becken und dem Ussuri-Gebiet noch in China, Korea und Japan verbreitet ist und westlich des Unterlaufes des Amurflusses nicht vorkommt.

Infolge der ungenügenden Kenntnis der Ephemeropterenfauna der UdSSR war unsere Vorstellung von der paläarktischen Fauna der Ephemeropteren überhaupt unvollkommen. Dieses hat das Studium einiger Familien besonders deutlich gezeigt. Das war der Fall bei der Entdeckung neuer Arten und Gattungen von Palingeniiden und Siphlonuriden, zahlreicher Arten von Ephemerelliden und bei der in der letzten Zeit gemachten Entdeckung einer eigenartigen Gattung der artenarmen Familie Behningiidae und einiger anderen. Diese neuen Angaben haben es möglich gemacht, die systematischen Beziehungen zwischen verschiedenen Gruppen genauer zu bestimmen. So, zum Beispiel, hat die Entdeckung einer Reihe von eigenartigen neuen Arten der Ephemerellidae gezeigt, daß die systematischen Beziehungen der paläarktischen Gattungen einer Revision unterworfen werden müssen.

Wie bekannt, ist den Ephemeropteren eine Reihe primitiver Merkmale eigen: Häutungen der geflügelten Phase, membranöse Flügel mit einer großen Anzahl von

Adern, Abdomen mit zehn Segmenten, mit langen Schwanzfäden und einem unpaarigen Paracercus, mit primitiven Genitalien usw. Die Larven der Ephemeropteren sind verschiedenartig gebaut und besitzen ausgesprochene Anpassungen an die verschiedensten Lebensbedingungen (darunter Anpassungen zu verschiedener Nahrungsaufnahme, zum Atmen in verschiedenen Wassertiefen, zum Leben im Boden, in Strömungen usw.). Es sind auch Beispiele der Entstehung von sehr ähnlichen morphologischen Strukturen bei den Vertretern verschiedener Gruppen der Ephemeropteren, die unter ähnlichen Bedingungen existieren, bekannt (z. B. gehören die Larven der Siphlonuridae und der Baëtidae zu zwei ähnlichen morpho-ökologischen Typen). Es ist das Entstehen von adaptiver Ähnlichkeit, die von der phylogenetischen Verwandtschaft wenig abhängig ist, bei der Feststellung der realen systematischen Beziehungen zwischen großen systematischen Einheiten zu berücksichtigen.

Es ist besonders wichtig, die Frage der Klassifikation der Larven und der geflügelten Phasen der Ephemeropteren zu streifen. Dieses wichtige Problem der Systematik entsteht jedesmal, wenn die Larven und die Imaginalphasen sich voneinander infolge verschiedener Lebensbedingungen stark unterscheiden. Für die Ephemeropteren ist die Lösung dieser Frage infolge der Reduktion der Imaginalphase, die nur sehr kurze Zeit existiert und sogar keine Nahrung aufnimmt, während die Entwicklung der Larve lang dauert, besonders wichtig. Diese Erscheinung läßt auf den größeren Wert der geflügelten Phasen für die Klärung der phylogenetischen Beziehungen schließen. Gerade die geflügelten Phasen der Ephemeropteren, als das reduzierte Lebensstadium in der historischen Entwicklung dieser Insekten, müssen bei der Feststellung der systematischen Beziehungen grundlegend sein. In zwei letzten Klassifikationsschemen der Ordnung (Edmunds-Traver, 1954, und Demoulin, 1958) beurteilen ihre Verfasser die Eigentümlichkeiten des Baues der Larven und der geflügelten Phasen verschieden, was sie zu verschiedenen Schlußfolgerungen über die Beziehungen der Gruppen führt. Ein gutes Beispiel dieser Meinungsverschiedenheiten kommt in der Einschätzung der Lage der Familie Neoephemeridae im System zum Ausdruck. Diese Eintagsfliegen sind, was den Bau der geflügelten Phase anbelangt, den Potamanthidae und Ephemeridae ähnlich, dagegen ist ihre Larve äußerlich den Larven der Vertreter der gut bekannten Gattung Caenis sehr ähnlich. Genannte Verfasser haben die Neoephemeridae zu verschiedenen Überfamilien zugerechnet. Ich habe mich speziell mit dieser Frage befaßt und habe festgestellt, daß die Neoephemeridae außer der Ähnlichkeit mit anderen Ephemeropteren auch eigenartige morphologische Eigentümlichkeiten besitzen, die es unmöglich machen, sie in die Überfamilie Ephemeroidea oder die der Caenoidea einzureihen. Die Neoephemeridae bilden eine selbständige Gruppe — eine besondere Überfamilie Neoephemeroidea.

Was das Klassifikationsschema von G. Demoulin anbetrifft, welches außer von Angaben über die rezente Fauna auch von paläontologischem Material ausgeht, so ist zu bemerken, daß letzteres erlaubt, verschiedene Ergänzungen zu diesem Schema zu machen. Es ist in der UdSSR zur Zeit ein umfangreiches Material von fossilen Eintagsfliegen vorhanden; sein Studium hat ermöglicht, eine Reihe von Schlußfolgerungen zu ziehen. Es wird wahrscheinlich notwendig sein, die von Demoulin vorgeschlagene Überfamilie der Oligoneurioidea, in der er jurassische Arten spezialisierten rezenten Oligoneuriidae und einigen anderen Gruppen nahe stellt, zu überprüfen. Wichtige Schlußfolgerungen lassen sich auch über die Beziehungen der jurassischen *Hexagenites*-Arten ziehen.

Das Studium der rezenten Ephemeropteren hat gezeigt, daß sie sehr gute Indikatoren der Futterstellen der Fische sind. Diese für die Praxis wichtige Schlußfolgerung ist das Ergebnis des Studiums des Darminhaltes von Fischen, die in verschiedenen Flüssen leben.

DIE TAXONOMISCHE SITUATION BEI DEN MITTELEUROPÄISCHEN EPHEMEROPTEREN

GERTRUD PLESKOT

Die letzte und zugleich einzige zusammenfassende Darstellung der Ephemeropteren Mitteleuropas stammt von Ulmer (1930, Tierwelt Mitteleuropas VI/3). Von den damals ca. 800 bekannten Arten behandelt er darin 86 Arten. Zur gleichen Zeit veröffentlichte Schoenemund eine Bearbeitung der Ephemeropteren Deutschlands (1930, Tierwelt Deutschlands, Teil 19), mit 66 Arten, in der erstmalig neben einem Bestimmungsschlüssel für die Imagines auch ein Artbestimmungsschlüssel für die Larven gegeben wird. Die Larven von 49 Arten werden beschrieben. Nur von einer Gattung ist die Larve unbekannt (*Ametropus*), nur für eine Gattung ist die Bestimmung der Larven aller Arten unmöglich (*Baetis*).

Seither sind in vielen Teilen Europas weitere Untersuchungen der Ephemeropterenarten durchgeführt worden, neue Arten wurden beschrieben, vor allem aber wurden manche der altbekannten Arten in allen Stadien exakter beschrieben und verglichen. Diese Bearbeitungen sind fast ausschließlich auf nationale Faunen bezogen. So fanden sich in Großbritannien, dem klassischen Ephemeropterenland Eaton's, Kimmins and Macan zur synchronen Bearbeitung der Imagines bzw. Larven des Landes (Literatur s. Referat Macan), womit 47 Arten taxonomisch geklärt und einwandfrei beschrieben erscheinen, von denen praktisch alle auch für Mitteleuropa in Frage kommen. In der ČSR setzt Landa die von Klapalek begründete Ephemeropterentradition fort (Literatur s. Referat Landa), in Österreich arbeitet Pleskot nach Brauer (Literatur s. Referat Pleskot), in Polen Keffermüller nach Mikulski, in Rumänien hat Bogoescu eine moderne Bearbeitung der dortigen Imagines veröffentlicht und zusammen mit Tabacaru die Beschreibung der Larven begonnen. Von den jugoslawischen Ephemeropteren wissen wir durch Ikonomov einiges, in Bulgarien hat Russev begonnen, die Landesfauna zu studieren. Die zahlreichen italienischen Untersuchungen über Ephemeropteren hat Grandi soeben in einem Buch zusammengefaßt. Dagegen fehlen aus West- und Nordeuropa zusammenfassende Darstellungen; nur für Finnland (Tienssu) und Norwegen (Brekke) gibt es einen faunistischen Überblick. Umfangreiche Studien über die russische Fauna betreibt Tschernova.

Die Großsystematik der Ephemeropteren wurde bisher in Europa wenig behandelt. Die Reihenfolge der Familien bzw. ihre Zusammenfassung zu Überfamilien ist in jedem Werk anders und wird nirgends begründet. (In neuester Zeit bearbeiten Traver und Edmunds in den Vereinigten Staaten — wo, begründet durch Needham, eine sehr intensive Ephemeropterenforschung betrieben wird — dieses Feld genauer. Auch Demoulin [Belgien] befaßt sich damit.) An der Gruppierung der Gattungen in Familien ändert sich dagegen sehr wenig. In letzter Zeit wurden auf Grund von neuentdeckten Formen einige neue Familien aufgestellt, die aber für Mitteleuropa nicht nachgewiesen sind. Die Adult-Stadien der merkwürdigen *Prosopistoma*-Larven wurden endlich, allerdings an außereuropäischem Material, nachgewiesen (Gillies, Fontaine).

Im Referat sind, dem Schlüssel von Ulmer folgend, die einzelnen Gattungen besprochen und ausführlich diskutiert worden. Hier sei nur einiges davon wiedergegeben.

Von den *Ephemera*-Arten, deren Imagines durchwegs gut beschrieben sind, sind nicht alle Larven bekannt, was ein Verständnis der Arten bzw. ihre biologische und ökologisch-geographische Vergleichung erschwert.

Die Abtrennung der Gattung *Habroleptoides* für die Art *modesta* Hagen durch Schoenemund, die von Bianchieri angegriffen wurde, erscheint mir sowohl taxonomisch (sehr

klare und konstante Merkmale sowohl der Imago wie der Larve) wie auch biologisch (deutliche Besonderheit dieser Art sowohl gegenüber den Leptophlebien wie gegenüber den Habrophlebien) sehr gut begründet, wie ich nach eingehendem Studium zahlreicher individuenreicher Populationen in österreichischen Gewässern glaube sagen zu können. Auch die wiederholt angezweifelte Trennung der *Habrophlebia*-Arten *fusca* Curtis und *lauta* McLachlan läßt sich auf Grund biologischer Befunde stützen. So scheint z. B. *fusca* niemals die großen auffälligen Schwärme zu bilden, die für *lauta* so charakteristisch sind. Die Bestimmung der Imagines beider Arten nach Einzelstücken mag allerdings manchmal (nach den bisher beschriebenen Unterschieden) zweifelhaft bleiben. Eine sichere Larvenbestimmung ist nach der Kiemenform (Landa 1957) immer möglich, wogegen der von Schoenemund beschriebene Unterschied in der Körperfärbung nur selten klar hervortritt.

Über *Paraleptophlebia Werneri* Ulmer sei mir eine faunistische Bemerkung gestattet. Die bisher nur einmal in Mitteleuropa (Niederösterreich) aufgefundene auffällige Art wurde 1952 von Adlmannseder im Gebiet der Antiesen (Oberösterreich) neuerlich nachgewiesen (17. 8. 1952, 4 ♂♂). 1960 entdeckte ich Ende Mai dichte Männchenschwärme im Tullnerfeld am Ufer langsamfließender durchwachsener Wassergräben, in deren Schlamm auch die bisher unbekannten Larven zu finden waren.

Dagegen konnte die von Brauer aus der Umgebung von Wien beschriebene Art *mesoleuca*, die nach Ulmer zu *Ephemerella* zu stellen ist, von uns bisher nicht wiedergefunden werden.

Wie Ulmer aufgezeigt hat, ist die Berechtigung einer Unterscheidung von zwei mitteleuropäischen *Torleya*-Arten (*major* Klp. und *belgica* Lest.) sehr fraglich: sie dürfte auf verschiedenartige Eintrocknung der Penisloben genadelter Exemplare zurückgehen! Bis zur Sicherung zweier Arten sollte daher wohl besser nur von *Torleya major* Klp. gesprochen werden.

Caenis ist eine von den Gattungen, für die die existierenden mitteleuropäischen Bestimmungsschlüssel nicht mehr verwendet werden können. Die entscheidende Revision ist beim Studium der britischen Fauna durch Macan und Kimmins gemacht worden. Die mitteleuropäische Fauna muß nun — ausgehend von diesen Beschreibungen sowohl der Imagines wie auch der Larven — neu bearbeitet werden. Alle früheren Artangaben sind nicht ohne weiteres verwertbar!

Dasselbe muß leider von den meisten Baetidenarten festgestellt werden. In der Gattung *Baetis* — seit jeher Schmerzenskind der Ephemeridentaxonomie — sind nur *pumilus*, *niger* und *atrebatinus* nach den beiden Standardwerken richtig bestimmbar (u. zw., da die Bestimmung nach Flügelmerkmalen erfolgt, sowohl Männchen wie Weibchen wie Subimagines). Für alle anderen Arten ist sowohl der Schlüssel von Ulmer wie der von Schoenemund unbrauchbar geworden. Die Bestimmung nach der Aderung des Hinterflügels läßt sich nicht durchführen und auch die anderen Merkmale ergeben in der verwendeten Kombination Fehlbestimmungen. Es sind daher die meisten älteren faunistischen Angaben über *Baetis*-Arten in Mitteleuropa nicht verwertbar!

Mehrere *Baetis*-Arten, die durch diese Schlüssel zum Teil zu den besonders oft zitierten zählen, sind taxonomisch oder differentialdiagnostisch nicht geklärt, z. B. *melanonyx* Pictet, *venustulus* Eaton und *nubecularis* Eaton. *B. melanonyx* dürfte — nach einer brieflichen Mitteilung von Kimmins, der 1957 Imagines der Eaton-Collection für mich untersuchte, und nach einer Bemerkung von Lestage (1917), daß nach Eaton die Larven dieser Art nur zwei Schwanzanhänge haben, ähnlich *alpinus* — der *alpinus*-Gruppe angehören, ist aber nach den derzeit vorliegenden Beschreibungen unbestimmbar. *B. venustulus* kann man wohl, zumindest vorläufig, als ein Synonym von *bioculatus* L. ansehen. Die Holotype ist im Britischen Museum verwahrt. Sie zeigt sowohl nach der allgemeinen Körperfärbung wie nach der Form der Genitalanhänge große Ähnlichkeit

mit *bioculatus* (Untersuchung und briefliche Mitteilung von Kimmins 1957) und wurde von Eaton zwischen *bioculatus* L. und *scambus Etn.* gestellt — zwei Arten, deren Trennung heute sehr schwierig und problematisch erscheint! Nach den Bestimmungsschlüsseln werden aber meist Imagines der *alpinus*-Gruppe als *venustulus* identifiziert werden. Auch die Trennung eines anderen Arten-Paares, *vernus* Curtis und *tenax* Eaton, ist vorläufig unsicher und fraglich. Die so häufig zitierte Art *gemellus* Eaton hat Kimmins (1960) als Synonym von *rhodani* Pictet erklärt und damit eliminiert! Viele *gemellus*-Angaben in der Literatur beziehen sich auf zweischwänzige Larven, was auf einen Irrtum von Lestage (1916) zurückgeht, der die von Steinmann (1909) gegebene Zeichnung des Hinterendes von *B. alpinus* Pictet übernimmt, aber als *gemellus* beschriftet. Durch das Werk von Rousseau (1921), das lange Zeit die einzige Zusammenfassung der europäischen Wasserinsekten war, ist diese Auffassung dann Allgemeingut geworden. Niemals aber wurde durch Aufzucht die Identität der betreffenden zweischwänzigen Larven (die oft zusammen mit *rhodani*-Larven die Gebirgsbäche bewohnen) mit *gemellus* festgestellt!

Die Larven der *Baetis*-Arten sind erst durch Macan bestimmbar geworden. Aus der *alpinus*-Gruppe („zweischwänzige" Larven), die in Großbritannien nicht vorkommt, hat Tabacaru einige Arten beschrieben. Die Arten dieser Gruppe verlangen aber dringend eine Revision.

Wie diese und verschiedene andere neue Arten (z. B. aus Italien von Grandi beschriebene), die differentialdiagnostisch noch nicht eindeutig eingereiht sind, zu beurteilen sein werden, muß erst noch erarbeitet werden. Offenbar sind bei den meisten Arten dieser Gattung die Imagines schwieriger zu unterscheiden als die Larven, so daß hier noch mehr als in anderen Fällen eine Klärung der offenen Fragen hauptsächlich vom Studium der Larven und von sorgfältiger Aufzucht des Adult-Materiales aus den Larven zu erwarten ist.

In der Gattung *Centroptilum* existieren neben den nun als Imago und Larve gut bekannten Arten *luteolum* Müller und *pennulatum* Eaton verschiedene noch mehr oder weniger ungeklärte Einzelbeschreibungen, die vermuten lassen, daß die Gattung doch mannigfaltiger ist.

Aus der Verwirrung in der Artbezeichnung bei *Cloeon* (die mit der fatalen Fehlbeschriftung einer Abbildung bei Eaton ihren Anfang nahm — s. Kimmins 1957) scheint nach neueren Untersuchungen die Zurückführung auf *dipterum* L. und *simile* Eaton (bzw. auf die Artenpaare *dipterum* — *inscriptum* Bengtsson und *simile* — *praetextum* Bengtsson) und auf eine als *Procloeon* abgetrennte Art *pseudorufulum* Kimmins herauszuführen. Doch wird hier nur eine sorgfältige Revision Klarheit schaffen können. Die mitteleuropäischen Bestimmungsschlüssel sind auch für diese Gattung nur sehr bedingt verwendbar. Der Larvenbestimmungsschlüssel in Schoenemund ist unverwendbar (vgl. Macan 1949, 1960).

Noch problematischer als bei den Baetiden ist die Lage in der Familie der Ecdyonuriden. Eine moderne Beschreibung der britischen Arten liegt auch hier vor, aber gerade in dieser Familie ist die britische Fauna auffallend artenärmer als die kontinentale, so daß noch viel zu tun bleibt, um die mitteleuropäische Artenliste klarzustellen.

In der Gattung *Epeorus* kann es vermutlich für Mitteleuropa bei den beiden sehr einfach trennbaren Arten *assimilis* Eaton und *alpicola* Eaton bleiben, nachdem die Diskussion *Epeorus-Iron* von den amerikanischen Untersuchern, die hiebei über das bessere Vergleichsmaterial verfügen, dahingehend entschieden wurde, daß *Iron* und andere Bezeichnungen in den Rang von Untergattungen gegenüber *Epeorus* verwiesen wurden (Edmunds-Traver 1954).

Die übrigen mitteleuropäischen Gattungen der Familie aber (*Rhithrogena*, *Ecdyonurus* und *Heptagenia*) sind derzeit in den meisten Arten ungeklärt.

Insbesondere die *Rhithrogena*-Arten sind heute weitgehend unbestimmbar und praktisch alle Literaturangaben über das Vorkommen von *Rhithrogena*-Arten sind zweifelhaft. Die für die Bestimmung maßgebliche Form der Penisloben ist durchwegs nur sehr ungenau (und oft nach getrocknetem Material) beschrieben. Die in Mitteleuropa nach meiner Erfahrung sehr verbreitete Art *semitincta* Pictet (von Kimmins gut beschrieben), die in den so häufigen Angaben von „*semicolorata*" steckt, ist in den Schlüsseln nicht enthalten. Alle *Rhithrogena*-Larven sind bisher unbestimmbar. Sie sind einander sehr ähnlich. Ihre Aufzucht zur Imago verlangt wegen der außerordentlichen Empfindlichkeit besondere Einrichtungen, die meist nicht zur Verfügung stehen. So kommt es, daß von den mitteleuropäischen Ephemeropteren *Rhithrogena* die vielleicht überhaupt am schlechtesten bekannte Gattung ist.

Bei *Ecdyonurus* ist die Situation nicht viel besser. Durch die sehr genaue Beschreibung der britischen *Ecdyonurus*-Arten in allen Stadien sind zwei mitteleuropäische Arten gut bekannt (*insignis* Eaton und *venosus* Fabricius). Die von Kimmins für Großbritannien neu beschriebene Art *torrentis* scheint nach meinen Erfahrungen in Mitteleuropa sehr verbreitet zu sein. Sie steht der Art *forcipula* Pictet sehr nahe. Ob *forcipula* selbst heute noch sicher reproduzierbar ist, scheint fraglich. Auch *fluminum* Pictet ist eine sehr schlecht charakterisierte Art.

Wichtig scheint es, darauf hinzuweisen, daß die Charakteristik der *forcipula*-Larven gegenüber *venosus*, die Schoenemund in seinem Schlüssel gibt, auf einem Irrtum beruht. Ich habe wiederholt aus solchen Larven *venosus*-Imagines gezogen. Es dürfte sich um eine Farbmutante handeln, die in *venosus*- (und *forcipula*- ?) Populationen in wechselnder Häufigkeit auftritt, aber nichts mit der Unterscheidung der beiden Arten *venosus* und *forcipula* zu tun hat. Die zahlreichen faunistischen Nachweise von *forcipula* sind also, soferne sie auf Larvenbestimmungen zurückgehen, nicht verwertbar.

Ecdyonurus helveticus Eaton wurde von Kimmins (1958) auf Grund von Material aus den österreichischen Alpen nach Vergleich mit dem Material der Eaton-Collection in drei Arten gegliedert: *austriacus* Kimmins, *helveticus* Eaton, *zelleri* Eaton. Sie unterscheiden sich eigentümlicherweise am deutlichsten durch die verschiedene Scheckung der Flügel im Subimagostadium. Das kurze erste Glied des männlichen Vordertarsus ist allen drei Arten gemeinsam.

Da es sich bei Baetiden und Ecdyonuriden um die verbreitetsten, häufigsten und zahlreichsten Ephemeropteren der meisten mitteleuropäischen Gewässer handelt, blockiert die taxonomische Unsicherheit in diesen beiden Familien vor allem viele ökologische Gewässeruntersuchungen. Auch in anderen Familien sind verschiedene Revisionen nach modernen Untersuchungsmethoden dringend notwendig. Es liegt in einem über den Bereich der Entomologie hinausgehenden allgemeinen Interesse, daß diese Unsicherheiten und Wissenslücken rasch beseitigt werden. Wenn dieses Symposium, das aus der Empfindung dieser Notwendigkeit heraus einberufen wurde, dazu ein Anstoß sein könnte, hätte es seinen Zweck erfüllt.

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DISKUSSION

V. LANDA: Frau Dozent Dr. G. Pleskot hat in ihrem Vortrag alle bisherigen Kenntnisse über einzelne Eintagsfliegenarten zusammengefaßt und verwertet und die Problematik angedeutet, die noch zu lösen ist. Ich möchte nur einige Anmerkungen und Ergänzungen beifügen, die sich aus dem Eintagsfliegenstudium in der ČSSR ergeben.

Palingenia longicauda (Oliv.) lebte am Anfange dieses Jahrhunderts in dem unteren Wasserlauf der March (Zavřel 1934). Heute ist sie schon von dieser Lokalität ganz verschwunden. Die Exemplare von diesem Fundorte, die sich in Klapáleks Sammlung befinden, entsprechen jenen aus der Donau, also der typischen Art *P. longicauda* (Oliv.), nicht aber der Art *P. sublongicauda*, welche Černova nach Exemplaren aus der USSR beschrieben hat. — In diesem Jahre hat man in der Ostslowakei ca. 10 Imagines der Art *Palingenia fuliginosa* Georgi gefangen.

Diese Art ist bisher nur vom Kaukasus bekannt. Ihr Fund in der ČSSR ist vom geographischen Gesichtspunkte sehr bedeutungsvoll. — *Ephemera lineata* Eaton tritt auf dem ganzen Gebiete der ČSSR auf und sie ist nicht selten. Ihre Larven bewohnen größere Flüsse mit sandigem und schlammigem Untergrund. — *E. vulgata* lebt in stehenden Wässern oder in den Buchten tieferer ruhig fließender Rinnsale. — Die häufigste Art, *E. danica* Müll., kommt in kleinen Bächen mit sandigem oder sandig-schlammigem Untergrund vor. — *Ecdyonurus fluminum* (Pictet) lebt als eine Sommerart in größeren Flüssen (Vltava, Berounka u. a.). Diejenigen Exemplare, welche Klapálek für diese Art hält, entsprechen der Art *E. zelleri* (Etn.). Diese fliegt in der ČSSR im Herbst in höheren Lagen.

Die Problematik weiterer Arten der *Ecdyonurus*-Gattung habe ich in meinem Referate während dieses Symposiums berührt. — *Heptagenia fuscogrisea* (Retz.) ist bei uns selten. *H. coerulans* (Rostock) ist von mehreren Lokalitäten bekannt. — Sehr häufig ist *H. affinis* (Eaton), eine Sommerart, die im August fliegt. Im Böhmerwalde wurde *Rhithrogena alpestris* (Etn.) gefunden. In der ČSSR kommen auch *Rh. haarupi* Esb. Pet., *Rh. germanica* (Etn.) und weitere häufige Arten dieser Gattung vor. Von mehreren Lokalitäten in Böhmen und in der Slowakei ist die Art *Arthroplea congener* Bengtsson bekannt. Ihre Larven leben am Schilf, am häufigsten in oligotrophen Teichen. — *A. frankenbergeri* Balthasar ist ein Synonymum von *A. congener* Bengtsson. — Häufig und sehr verbreitet ist die Art *Siphlurella linnaeana* (Eaton). *Isonychia ignota* (Walker) wurde in der Donau gefunden. — *Ameletus inopinatus* Eaton bewohnt Bergbäche und Wasserbehälter unserer Grenzgebirge. Häufig kommt sie in den Tatraseen vor; die in den Seen vorkommenden Exemplare sind ziemlich größer, aber ohne deutlich morphologisch verschiedene Merkmale.

Ich bin ganz der Ansicht von Kollegin Pleskot, daß die Arten *Baëtis bioculatus* (L.) und *B. scambus* Eaton an einer Seite und *B. vernus* Curtis und *B. tenax* Eaton an anderer Seite identisch sind. Diese Arten sind schon deshalb variabel, weil sie zwei Generationen haben und weil sie, besonders *B. vernus* Curtis, in ziemlich großer Höhenbreite leben. Meine Exemplare, die mehr der Art *B. tenax* Eaton entsprechen, sind Herbstarten und größtenteils von höheren Lagen. Völlig stimme ich auch der Ansicht von Pleskot zu, daß *B. gemellus* Eaton eine sehr problematische Art ist. Ich meine, daß hier Exemplare der zweiten Generation von *B. rhodani* (Pictet) von hohen Lagen beschrieben wurden. In der ČSSR wurde *B. gemellus* Eaton häufig von Bergwasserläufen angeführt, und zwar nach ihren Larven mit dem verkümmerten Mittelschwanzfaden. Rezente Untersuchungen haben aber gezeigt, daß es sich in allen Fällen um *B. alpinus* (Pictet) handelte. *B. alpinus* (Pictet) ist eine Art, die in den Bergen Mitteleuropas weit verbreitet ist. Es wird notwendig sein, alle beschriebenen Bergarten mit dem langen Endglied der Gonopoden (*B. carpathicus* Morton, *B. culindrophthalmus* Bogoescu u. a.) eingehender zu vergleichen. Es handelt sich hier sehr wahrscheinlich um dieselbe Art in verschiedenen geographischen Rassen. *Centroptilum pennulatum* Eaton ist im ganzen selten, befindet sich aber auf mehreren Fundorten. Häufiger tritt *Procloeum pseudorufulum* Kimmins auf. *Cloëon praetextum* Bengtsson wurde in der ČSSR nicht gefunden. Die Taxonomie dieser Art braucht noch weiteres Studium. Ich bemerke, daß auch die Larven und Subimagines, die ich in Island gesammelt habe und die für diese Art betrachtet werden, sich von der Originalbeschreibung wesentlich unterscheiden. — *Paraleptophlebia cincta* (Retzius) ist eine typische Sommerart und ist an mehreren Lokalitäten sehr häufig. Auch Larven und Imagines der Art *Paraleptophlebia tumida* Bengtsson wurden in einigen Stücken gefunden. *Choroterpes picteti* (Eaton) ist eine verhältnismäßig häufige Art. Ich bin der Meinung, daß es nicht richtig ist, die Gattungen *Habroleptoides* und *Habrophlebia* zusammenzuziehen. Die Larvenmerkmale, wie die äußerliche morphologische, so auch die anatomische (die Bildung des Tracheensystems) sprechen für solches Zusammenziehen nicht. — *Ephemerella notata* Etn. ist im Gegenteil zur *E. ignita* Poda eine typische Winterart. Sie fliegt im Frühling aus. Sie wurde an 5 Stellen in Böhmen und in Mähren (in der westlichen Hälfte der ČSSR) gefunden. Es ist interessant, daß von 370 Larven, Subimagines und Imagines kein einziges Exemplar Männchen war. Es handelt sich hier wohl um eine geographische Parthenogenese. — Ich bin der Meinung, daß es richtig wäre, die Gattung Chitonophora der Gattung *Ephemerella* anzuschließen. Wenn wir nämlich paläarktische und nearktische Arten der Gattung *Ephemerella* vergleichen, so sehen wir, daß die Merkmale der Gattung *Chitonophora* sich gänzlich mit den für die Gattung *Ephemerella* charakteristischen Merkmalen decken. — Auf Grund meines Materials meine ich, daß die Art *Torleya belgica* Lest. mit der Art *T. major* Klapálek identisch ist. — Eine der häufigsten Arten der Gattung *Caenis* in der ČSSR ist die Art *Caenis robusta* Etn. Sie hat 2 Generationen und kommt wie in stehenden so auch in fließenden Wässern vor. Sie ist offensichtlich in ganz Mitteleuropa stark verbreitet. — In Mitteleuropa ist ferner auch die Art *C. undosa* Tiensuu verbreitet, die ursprünglich nur von Norden bekannt wurde. — An mehreren Lokalitäten wurden die Arten *Brachycercus harrisella* (Curtis) und *Caenis moesta* Bengtsson gefangen. — *Prosopistoma foliaceum* (Fourcroy), die im Jahre 1923 in der Moldau in Prag von Prof. Klapálek entdeckt wurde, wurde seither nicht mehr gefunden.

DIE ENTWICKLUNG DER MITTELEUROPÄISCHEN EPHEMEROPTEREN

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Im Zusammenhang mit dem Studium der wichtigsten Fragen der Reinheit der Wasserläufe werden in den letzten Jahren in der ČSSR sehr ausführliche Forschungen über die Verbreitung und Saisondynamik der Wasserinsekten durchgeführt, darunter natürlich auch der Ephemeropteren. Die Forschungen werden nach und nach in einzelnen Gebieten durchgeführt. In jedem Gebiet (Böhmen wurde z. B. in 6 solche Gebiete eingeteilt) wurden einerseits ständige Lokalitäten untersucht, anderseits solche, die nur einmal, zweimal bis dreimal während des Jahres besucht wurden. Die ständigen Lokalitäten, von denen sich in jedem Gebiete 10—15 befinden, werden regelmäßig monatlich vom Februar bis November besucht. Die erworbenen Daten dienen zur Erkenntnis der Saisondynamik und der Entwicklung der Arten. Die anderen Lokalitäten, von denen sich in jedem Gebiete etwa 100—150 befinden, dienen zur Kenntnis der Verbreitung der einzelnen Arten. Die Durchforschung jedes Gebietes dauert 2 Jahre. Es werden wie die Larven so auch die Imagines und Subimagines gesammelt. Von jedem Gebiete werden etwa 10.000—15.000 Exemplare gewonnen. Diese Durchforschung der ČSSR wird voraussichtlich im Laufe von 3 Jahren beendigt sein. Sehr reiches, regelmäßig gesammeltes Material und die entsprechende Züchtung im Laboratorium hat schon heute eine gute Übersicht über die Entwicklung mitteleuropäischer Eintagsfliegenarten gegeben.

Die mitteleuropäischen Eintagsfliegen haben am häufigsten einjährigen Entwicklungszyklus. Sehr wichtige Rolle spielt hier die Ei-Diapause bzw. die Diapause von jungen Larven. Eigentliches Wachstum älterer Larven (d. h. der Larven vom 10.—12. Stadium ab, die schon vollständig entwickelt sind und die die Flügelscheiden anzusetzen beginnen), welche wir bei normaler Gewässeruntersuchung finden, ist deshalb viel kürzer.

In der einjährigen Eintagsfliegenentwicklung kann man folgende 3 Haupttypen feststellen:

1. Ältere Larven entwickeln sich — zwar langsamer — auch im Winter und ihr Wachstum dauert 6—9 Monate. Sie können „Winterarten" genannt werden. Zu diesen gehört z. B. *Epeorus assimilis* Etn., *Rhithrogena semicolorata* (Curt.), *Rhithrogena germanica* Etn., *Rhithrogena haarupi* Esb. Pet., *Ecdyonurus torrentis* Kimmins, *Heptagenia flava* (Rost.), *Heptagenia sulphurea* (Müll.), *Heptagenia lateralis* (Curt.), *Leptophlebia marginata* (L.), *Leptophlebia vespertina* (L.), *Paraleptophlebia submarginata* (Steph.), *Habroleptoides modesta* (Hag.), *Ephemerella krieghoffi* (Ulm.), *Ephemerella notata* Etn., *Torleya maior* Klap. u. a.

2. Ältere Larven entwickeln sich schnell in den Sommermonaten, ihre Entwicklung dauert höchstens 3 Monate, gewöhnlich nur 2 und weniger. Den größten Jahresteil überdauern diese Arten in Ei-Diapause. Solche Arten sind: *Oligoneuriella rhenana* (Imhoff), *Polymitarcis virgo* (Oliv.), *Arthroplea congener* Bgtss., *Rhithrogena aurantiaca* (Burm.), *Rhithrogena alpestris* (Etn.), *Ecdyonurus insignis* (Etn.), *Ecdyonurus fluminum* (Pict.), *Ecdyonurus dispar* (Curt.), *Heptagenia affinis* (Etn.), *Siphlurella linnaeana* (Etn.), Arten der Gattung *Siphlonurus*, *Ameletus inopinatus* (Etn.), *Choroterpes picteti* (Etn.), *Ephemerella ignita* (Poda), *Caenis undosa* Tiensuu u. a. Die Sommerarten sind — allgemein gesprochen — sehr variabel.

3. Das eigentliche Wachstum der älteren Larven dauert eine kurze Zeit, nicht länger als 3 Monate. Die Larven schlüpfen schon im Herbst und überdauern den Winter und die Frühlingsmonate in Diapause, das eigentliche Wachstum kommt in den Sommermonaten zustande. Hierher gehören die Arten der Gattung *Habrophlebia*, *Potamanthus luteus* (L.), vielleicht noch weitere Sommerarten.

Eine ganze Reihe unserer Arten hat in einem Jahre 2 Generationen. Es sind meist die Arten der Gattung *Baëtis*, *Cloëon*, *Centroptilum*, *Procloëon*, die Mehrzahl der Arten

von der Gattung *Caenis* und die Art *Ecdyonurus subalpinus* Klap. Die ersten Generationen dieser Arten verhalten sich wie die „Winterarten", die zweiten Generationen wie die „Sommerarten". Untersuchungen in den Teichen bei Lnáře (Südböhmen) zeigen, daß einzelne Arten, z. B. *Caenis horaria* (L.) und *Cloëon dipterum* (L.) im Laufe eines Jahres unter günstigen Bedingungen auch 3 Generationen erzeugen können. Bei den Arten, die in einem Jahre 2 Generationen haben, kann der Fall vorkommen, daß sich 3 Generationen im Laufe von 2 Jahren entwickeln. Zu dieser Situation kommt es dann, wenn die zweite Generation unter ungünstigen Bedingungen sich tief in den Herbst hinein verschiebt. Falls auch im Frühling nicht besonders günstige Bedingungen vorkommen, so verschiebt sich die erste Generation in den Sommer hinein. Die zweite Generation fliegt nicht mehr aus, sie überwintert und im dritten Jahre fliegt sie früh aus, so daß auch die zweite Generation sich rechtzeitig entwickelt. Dieser Fall ist bei der amerikanischen Art *Baëtis vagans* McDunn. (Murphy, 1922) und bei den europäischen Arten *Cloëon dipterum* (L.), *Centroptilum luteolum* (Müll.), *Cloëon simile* Etn. (Degrange, 1960) beschrieben. Ich selbst habe diesen Fall mehrmals bei *Baëtis pumilus* (Burm.), *Cloëon simile* Etn., hauptsächlich aber bei *Baëtis rhodani* (Pict.) beobachtet. Zum Unterschied von den amerikanischen Autoren betrachte ich 3 Generationen nicht als regelmäßige Erscheinung, sondern als einen durch ungünstige Bedingungen verursachten Ausnahmefall. Den zweijährigen Entwicklungszyklus habe ich ganz sicher bei den Larven von *Ephemera danica* Müller und *Ephemera vulgata* Linné beobachtet. Er wird wahrscheinlich auch bei den anderen 2 Arten dieser Gattung vorkommen. Die Flugzeit dieser Arten ist vom April bis zum September verlängert. Auch der Fall von 3 Ausflügen in zwei Jahren ist hier möglich. Nach Literaturangaben ist die mehrjährige Entwicklung bei der Art *Palingenia longicauda* Oliv. sicher festgestellt worden.

Die Entwicklungszeiten der älteren Larven einzelner Arten sind über das ganze Jahr verteilt. In der Natur ist es nämlich so, daß in den Sommermonaten einerseits die Sommergeneration einiger Arten, anderseits die Sommerarten auftreten. Es ist merkwürdig, daß manchmal zwei verschiedene Arten einer Gattung an denselben Lokalitäten vorkommen: eine Winterart und eine Sommerart. Ich denke, daß es möglich sei, daß aus einer ursprünglichen Sommergeneration einer Art eine neue Art im Laufe der Entwicklung entstehen konnte. In dieser Hinsicht sind besonders die Verhältnisse bei der Gattung *Ecdyonurus* interessant. In Mitteleuropa ist die Art *Ecdyonurus torrentis* Kimmins sehr verbreitet. Exemplare dieser Art leben an verschiedenen Biotopen, hauptsächlich in den Bächen, vom August bis Mai. Im Mai fliegen die Imagines. Im Sommer kommen an denselben Lokalitäten andere Larven vor, die sich rasch in zwei Monaten entwickeln. Imagines fliegen dann im Herbst, und zwar im September. In den niedrigeren Lagen entsprechen die Larven und Imagines dem *Ecdyonurus dispar* Curt., in den höheren Lagen sind sie dieser Art sehr nahe. Selbstverständlich sind diese Sommerarten sehr variabel. Eier dieser Sommerarten diapausieren bis zum nächsten Jahr. In den größeren Flüssen, wo die Entwicklung von *E. torrentis* verlangsamt, die Entwicklung von *E. dispar* dagegen beschleunigt wird, entwickeln sich die Larven dieser zwei Arten fast gleichzeitig. Man kann voraussetzen, daß es sich um bisher nicht genügend stabilisierte Arten handelt, die von der Sommergeneration der *E. torrentis* ursprünglich abzuleiten sind. Diese Frage wird in der Tschechoslowakei noch weiter studiert.

VERZEICHNIS

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EXPERIMENTE ZUR ATMUNGSPHYSIOLOGIE VON EPHEMEROPTEREN-LARVEN

AFAF MOHAMED HILMY

(Siehe Tafel VII)

Die Auffassungen über die Bedeutung und Funktionsweise von Tracheenkiemen und anderen Atmungsvorrichtungen des geschlossenen Tracheensystems sind uneinheitlich, jedoch sind nur wenige Experimente zu der Frage durchgeführt worden.

In letzter Zeit haben Beobachtungen von Harnisch, Wigglesworth, Beier, Metzky, Ruß, Balke diese Fragen erneut zur Diskussion gebracht.

Da die Atmungsbedingungen in Gewässern häufig zum Minimumfaktor für das Gedeihen von Insekten werden, ist die Kenntnis der atmungsphysiologischen Anforderungen an das Milieu für die biologisch-ökologische Arbeit mit Wasserinsekten besonders wichtig.

Hier soll ein Abriß der Ergebnisse von Experimenten mit Ephemeropteren-Larven gegeben werden, die in Wien und in Lunz, Niederösterreich, durchgeführt wurden. Es wurden zwei Leptophlebiiden (*Habroleptoides modesta* Hagen und *Habrophlebia lauta* McLachlan) und eine Ephemerellide (*Ephemerella ignita* Poda) beobachtet und sowohl die Sauerstoffaufnahme wie die Kohlendioxyd-Abgabe kontrolliert.

Zwei Fragestellungen wurden behandelt: 1. Die Abhängigkeit der absoluten Größe des Gasaustausches vom Entwicklungsstadium und von der Wassertemperatur. 2. Die Lokalisierung der Gasaustauschstellen.

Das erste Problem wurde so untersucht, daß je 15—60 Tiere vom gleichen Stadium in eine verschlossene Flasche gebracht wurden und nach einer Exposition von jeweils $^1/_2$ bis 2 Stunden bei konstanter Temperatur zuerst der CO_2-Zuwachs mit der Klut'schen Methode und dann mit denselben Tieren der O_2-Verbrauch nach Winkler bestimmt wurde. Mittels des Trockengewichtes der Tiere wurde für jeden Versuch die Gasmenge pro Gramm, Tier und Stunde berechnet. Die einzelnen Versuche wurden 6—10mal mit jeweils anderen Tieren wiederholt. Die Versuchstiere wurden vor den Experimenten einige Tage im Laboratorium bei gleichmäßiger Temperatur und normaler Ernährung (Detritusfresser) gehalten.

Die CO_2-Abgabe wurde auch mit einer radiochemischen Methode (Verfütterung von C_{14}-hältigem Detritus und Bestimmung der Radioaktivität des abgegebenen CO_2) gemessen.

Die vollständigsten Ergebnisse wurden bei *Habroleptoides* erzielt. Die absoluten Werte der Sauerstoff-Aufnahme schwankten bei dieser Art zwischen 1,8 (erwachsene Nymphen, 5°C) und 9,0 (junge Nymphen, 20°C) mg O_2/g Tier und Stunde, die der CO_2-Abgabe zwischen 2,3 und 10,0 mg CO_2. Die entsprechenden Werte bei *Habrophlebia* und *Ephemerella* sind der Tabelle 1 zu entnehmen. Die radiochemische Messung ergab für *Habroleptoides* ungefähr dieselbe Größenordnung der CO_2-Abgabe, aber eine geringere Schwankung mit dem Entwicklungsstadium: 4,0—6,6 mg CO_2/g Tier und Stunde.

Tabelle 1

Niedrigste und höchste Werte der O_2-Aufnahme und CO_2-Abgabe bei den Versuchstieren

	Grenzwerte pro Gramm, Tier und Stunde	
	O_2-Aufnahme	CO_2-Abgabe
Habroleptoides modesta	1,8— 9,0	2,3—10,0
Habrophlebia lauta	1,7— 7,3	1,9— 8,7
Ephemerella ignita	3,0—11,2	3,2—11,4

Die wechselnde Intensität des Gasaustausches von *Habroleptoides*-Nymphen in den einzelnen Stadien und bei den verschiedenen Temperaturen ist in Abb. 1 wiedergegeben.

Die Temperatur wurde zwischen 5 und 25°C variiert. In allen Stadien tritt ein Knick in der Kurve bei 20—22°C auf, mit einer starken Senkung der Werte bei 25°C. Im übrigen Temperaturbereich entspricht der Verlauf der Kurve etwa der van t'Hoff'schen Regel.

Die Relation zwischen den Werten der einzelnen Stadien bleibt im wesentlichen bei allen Temperaturen die gleiche: Den intensivsten Gasaustausch zeigen die jungen Nymphen, die Werte sinken über die halbwüchsigen zu den erwachsenen Nymphen auf ein Minimum, steigen aber bei der zum Schlüpfen reifenden Nymphe sprunghaft wieder an, wobei sie in der Regel den Wert der jungen Nymphe nicht ganz erreichen. In Tabelle 2 ist das Maß dieser Relationen quantitativ wiedergegeben.

„Junge Nymphen“ sind allerdings relativ weit entwickelte Stadien mit bereits vorhandenen kurzen Flügelscheiden. Über den Gasaustausch der jüngsten Stadien (Eilarven, Larvulae und Larven ohne Flügelscheiden) wurden noch keine Experimente angestellt.

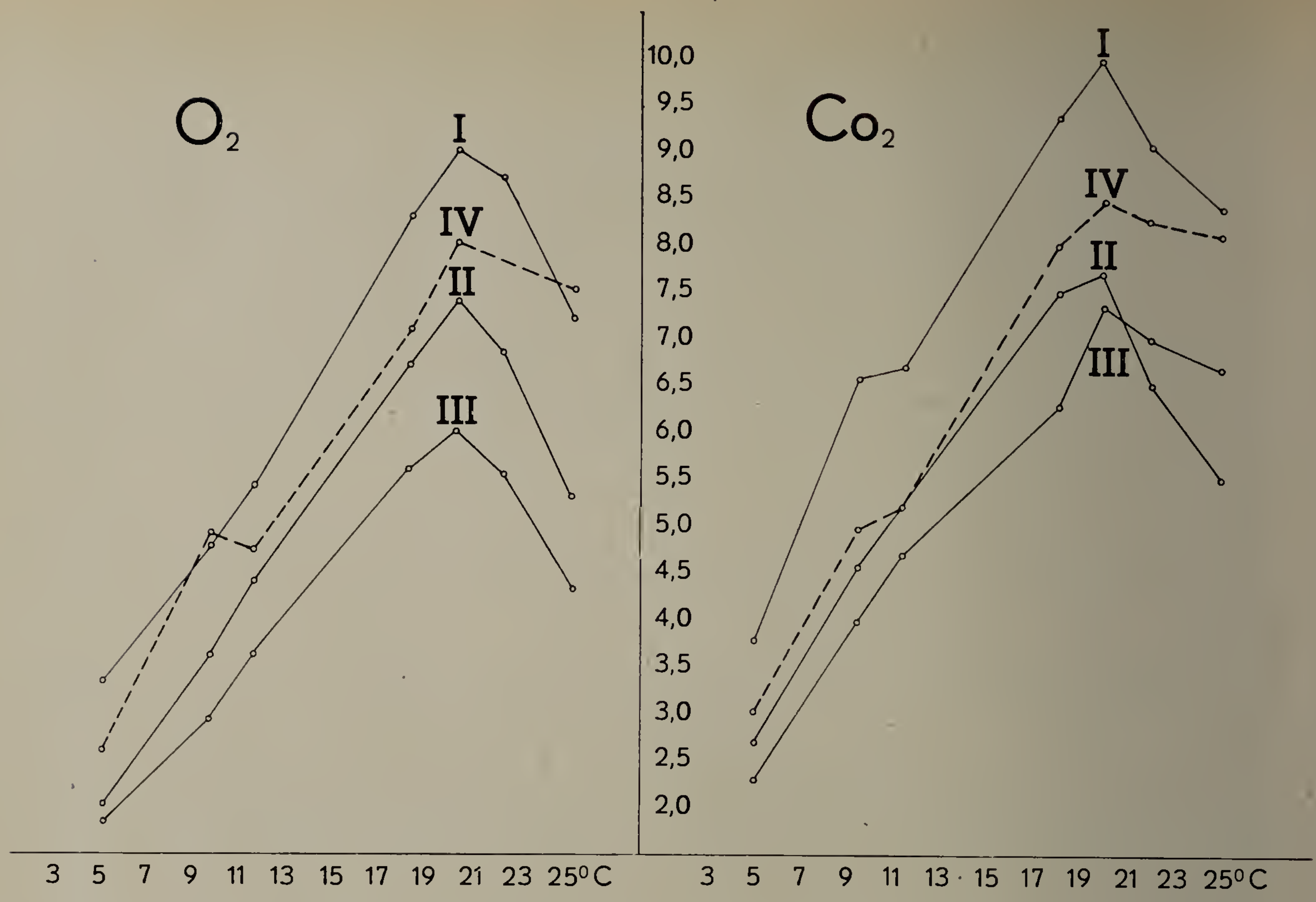

Abb. 1. *Habroleptoides modesta.* Temperaturabhängigkeit der O_2-Aufnahme und CO_2-Abgabe bei jungen (I), halbwüchsigen (II), erwachsenen (III) und reifen (IV) Nymphen.

Tabelle 2

Habroleptoides. O_2-Aufnahme und CO_2-Abgabe verschiedener Entwicklungsstadien in Prozent des Wertes der jüngsten Tiere

bei °C		I junge Nymphen absolute Werte mg $O_2(CO_2)$/g je Tier u. St.	II halbw. Nymphen	III erw. Nymphen	IV reife Nymphen
			relative Werte % von I		
5,0	O_2	3,3	60	54	78
	CO_2	3,8	74	60	79
9,5	O_2	4,7	76	62	104
	CO_2	6,6	83	60	76
11,4	O_2	5,4	82	66	87
	CO_2	6,7	77	70	79
18,0	O_2	8,3	81	54	85
	CO_2	9,4	80	67	85
20,0	O_2	9,0	82	67	89
	CO_2	10,0	77	74	85
22,0	O_2	8,7	78	63	90
	CO_2	9,1	72	77	91
25,0	O_2	7,2	74	60	104
	CO_2	84	65	79	95

AFAF MOHAMED HILMY:

Experimente zur Atmungsphysiologie von Ephemeropteren-Larven

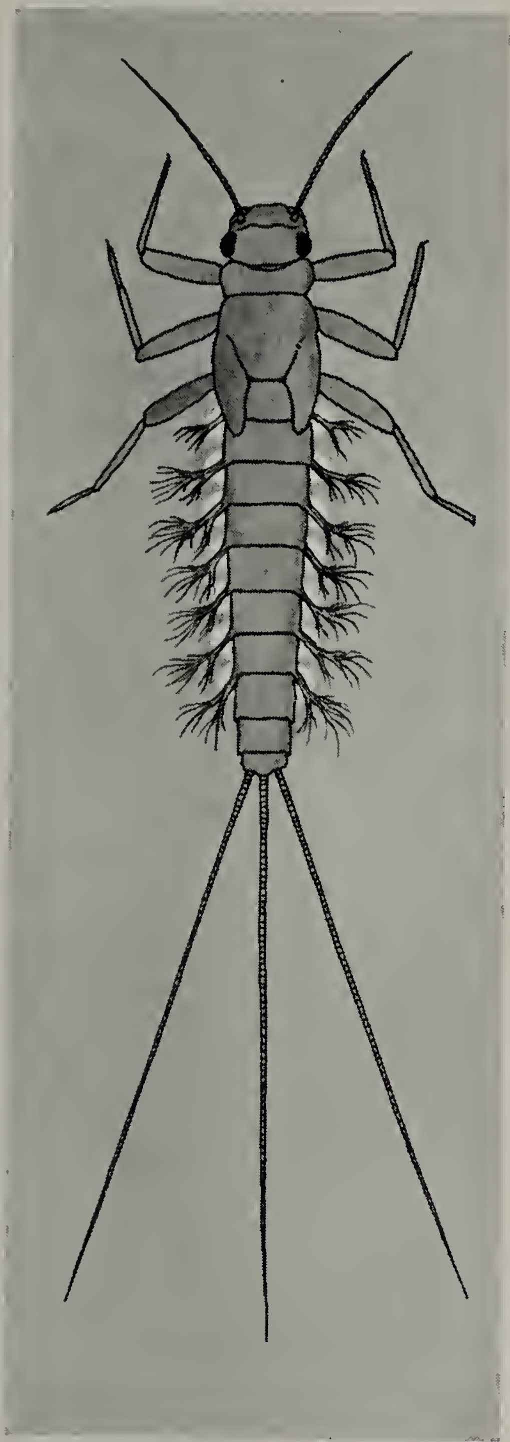

Abb. 3

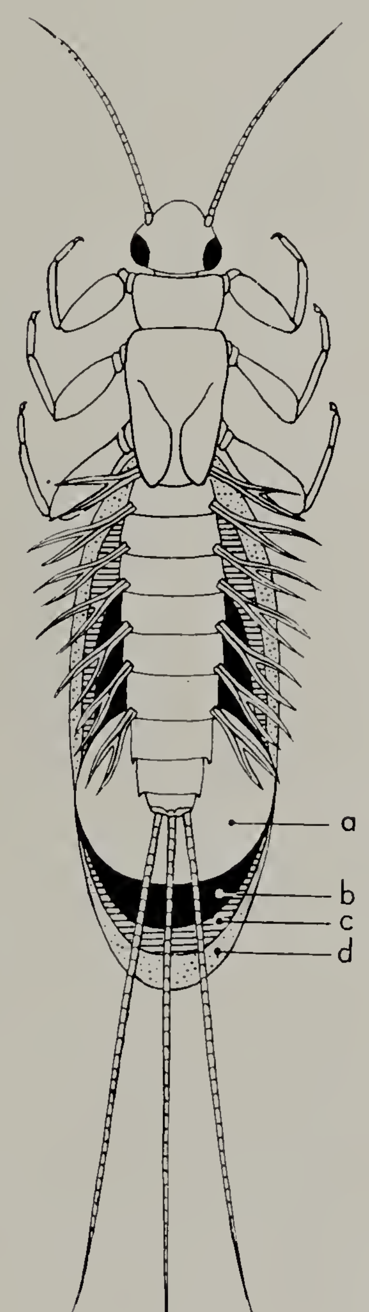

Abb. 4

Abb. 5

Abb. 3. *Habrophlebia lauta*. Entfärbungsbild, wenige Sekunden nach dem Einbringen der Larve in Phenolphthalein. CO_2-Abgabe im basalen Teil der Kiemen.

Abb. 4. *Habroleptoides modesta*. Zeitliche Ausbreitung des Entfärbungsbildes während einer halben Stunde. CO_2-Abgabe vom Abdomen, mit abnehmender Intensität gegen das Vorderende hin. Entfärbungsbild nach 5 (a), 10 (b), 15 (c) und 30 (d) Minuten.

Abb. 5. Tracheenkieme von *Habroleptoides modesta* nach Schwärzung mit Silbernitrat.

1 = geschwärzte Tracheenhauptstämme,
2 = Tracheolen,
3 = Chitinporen.

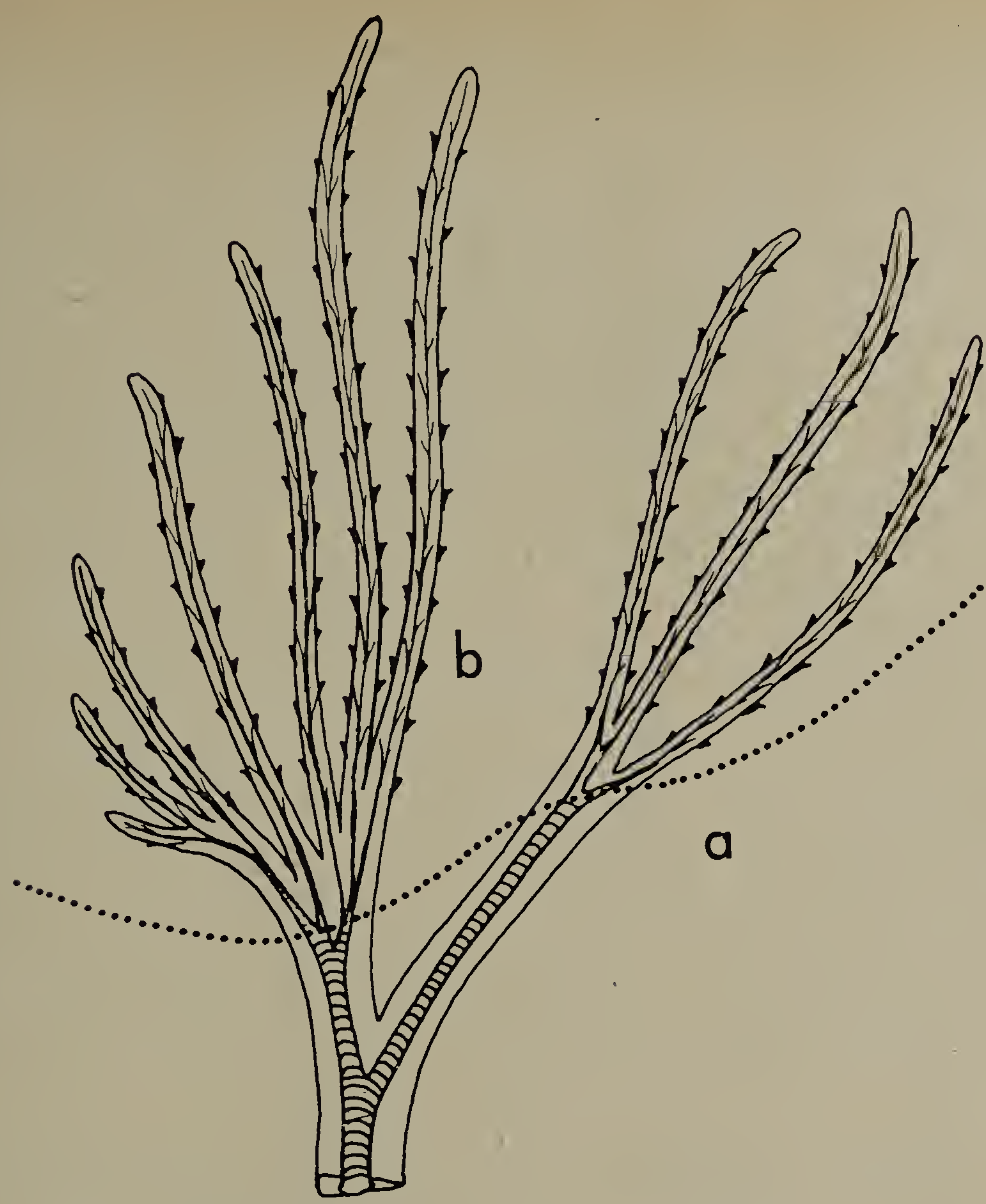

Abb. 2. Tracheenkieme von *Habrophlebia lauta*.
a = basaler Abschnitt mit den versteiften Haupttracheenstämmen (Bereich der CO_2-Abgabe).
b = distaler Abschnitt mit den verästelten, unversteiften Tracheenästen und den Tracheolen (Bereich der O_2-Aufnahme).

Jedenfalls zeigen die Ergebnisse, daß nicht nur die Temperatur den Atmungsvorgang in der bekannten Weise beeinflußt, sondern daß auch das Entwicklungsstadium für die atmungsphysiologischen Ansprüche an das Milieu ausschlaggebend ist und mit berücksichtigt werden muß. Als besonders anspruchsvoll erwiesen sich erwartungsgemäß die Stadien mit dem stärksten Wachstum (junge Nymphen) und die Stadien mit den intensivsten Metamorphosevorgängen (reife Nymphen).

Das zweite Problem, die Lokalisierung der Gasaustauschstellen, wurde mit einfachen Vitalfärbungsmethoden untersucht (Silbernitratreduktion an O_2-Aufnahmestellen bei Lichteinwirkung bzw. Phenolphthalein-Entfärbung an CO_2-Abgabestellen). Das überraschende Ergebnis war, daß bei zwei so nahe verwandten Formen wie *Habroleptoides* und *Habrophlebia* völlig verschiedene Verhältnisse vorliegen.

Durch die Beobachtungen von Metzky (Coleopteren) und Ruß (Dipteren) war es wahrscheinlich geworden, daß bei Tracheenkiemenatmung die O_2-Aufnahmeorte von den CO_2-Abgabeorten getrennt liegen.

Das ist auch bei *Habrophlebia* der Fall. Die Situation bei dieser Art entspricht völlig der Erwartung. Die vielästig aufgespaltenen Tracheenkiemen (Abb. 2) sind im Basalteil von unverzweigten, spiralig versteiften Haupttracheenästen durchzogen, während ihre Verästelung in feinste, unversteifte Äste und Tracheolen auf die zarten Spitzenteile beschränkt ist. Bei Einwirkung von Silbernitrat färben sich ausschließlich diese Spitzenteile schwarz, bei Einlegen des Tieres in Phenolphthalein (Abb. 3) entfärbt sich zu allererst die Umgebung der Tracheenkiemen-Basis.

Der Sauerstoff wird also bei *Habrophlebia* in dem tracheolendurchsponnenen distalen Abschnitt der Kiemen aufgenommen, während die stärkste CO_2-Abscheidung an der Kiemenbasis erfolgt.

Bei *Habroleptoides* dagegen liegen die Dinge wesentlich anders. An der Phenolphthalein-Entfärbung (Abb. 4) nehmen die Tracheenkiemen überhaupt keinen Anteil. Die Entfärbung tritt am schnellsten und intensivsten in der Umgebung des Hinterendes ein und breitet sich von hier allmählich über das ganze Abdomen aus. Mit Silbernitrat tritt an keinem Abschnitt der Kiemen eine Schwärzung der Kutikula ein. Dagegen färben sich die Chitinporen, die hier die Kiemen ziemlich gleichmäßig bedecken (Abb. 5) schwarz und zugleich die Tracheenhauptstämme, die hier beide Äste der Kieme bis in die Spitze unverästelt durchziehen und an der ganzen Länge von der Basis der Kiemen bis zu den Spitzen der Gabeläste ein dichtes Netz fast radiär gestellter kurzer Tracheolen zur Oberfläche senden. Die Chitinporen liegen deutlich zwischen den Tracheolen und es sieht an den Totalpräparaten nicht so aus, als ob eine Verbindung zwischen beiden bestehen würde (Schnitte wurden nicht angefertigt).

Die Chitinporen, die von Schoenemund, Prey und Kühnelt bei verschiedenen Insekten beschrieben wurden, sind vorläufig rätselhafte Organe. Auch Kühnelt hat beobachtet, daß mit der Schwärzung der Poren eine Schwärzung der Tracheenstämme Hand in Hand geht, während die Kutikula ungeschwärzt bleibt. Eine funktionelle Deutung dieser Vorgänge ist vorläufig nicht möglich, doch kann eines sicher gesagt werden: Die Funktionsweise der Tracheenkiemen von *Habroleptoides* unterscheidet sich offensichtlich irgendwie sehr entscheidend von der bei *Habrophlebia!*

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EPHEMEROPTERA IN BRITAIN

T. T. MACAN

Forty-seven species have been recorded from Britain, and recent taxonomic studies are set out in the reference list.

The status of *Arthroplea congener* on the British list, being based on one specimen taken 40 years ago, is somewhat doubtful. *Ephemera lineata*, *Potamanthus luteus*, *Brachycercus harrisella*, *Baëtis atrebatinus*, and perhaps also *Rhithrogena haarupi*, are rare species inhabiting what in Britain pass for large rivers. Their range has possibly been reduced by man's use of rivers for a variety of purposes. *Heptagenia fuscogrisea* is common in the limestone lakes in Ireland but rare in the rest of the British Isles. It is the only

ecdyonurid I have ever found clinging to flat leaves. *Paraleptophlebia tumida* is known to me only from two chalk streams that are dry in summer. *Ameletus inopinatus*, a mountain and arctic species in Europe, is abundant in streams above 300—400 m though I have also found it in lakes at sea level in the north of Scotland.

Accounts of life histories have been published by Harker (1952), Macan (1957) and Gledhill (1959). Once a life history has been worked out, there is the problem of finding which internal or external factors cause the various events to happen when they do. Adults of *Rhithrogena semicolorata* were taken in an emergence cage up to late August in 1953, 1954, and 1956, three summers notable for persistently low temperature. In four other years emergence ended when the average air temperature, which is followed fairly closely, though with some lag, by water temperature, rose to 16—17°C and stayed there for at least a week (Macan 1960a). A destruction by lethally high temperature of nymphs that have not emerged seems likely. It is impossible to say what the actual lethal level is from the figures quoted; not only temperature reached but duration of exposure is important. As will be described presently, these small streams may be as warm in late May as at any time in the summer, but in this month they are colder at night than in July or August (Macan 1959). Whitney (1939) found a lethal temperature of 22.4—24.7°C but the value is likely to be lower during the critical period just before emergence (Pleskot 1953). Presumably the eggs can withstand a higher temperature than the nymphs, support for which idea comes from a comparison of the results of Harker (1952) and myself (Macan 1957). Harker found that emergence lasted well into July and was not over before the nymphs of the new generation appeared, whereas in my stream emergence was mainly in June or late May, the tiny nymphs never appeared before August, and the two yearclasses never overlapped. The later emergence and earlier hatching in Harker's stream is probably correlated with its lower temperature.

Heptagenia lateralis has a life history which is very like that of *Rhithrogena semicolorata* and shows the same difference in the two streams studied by Harker and myself. It differs from it in two respects, both of which restrict its range: it does not grow during the coldest part of the winter, whereas *Rhithrogena* increases in size at a steady rate regardless of temperature, and its upper lethal temperature is lower (Macan 1960b). In 1959, a summer with an unusually long duration of sunny weather, the maximum temperature at 18 stations in streams was measured at intervals. Where it was 18°C or less, *Heptagenia* was always numerous, and where it was 18°C or higher, *Heptagenia* was scarce or absent except at one station, which, however, was just above a particularly thickly populated cool zone. *Heptagenia* occurs also on the stony shores of Windermere, which gets several degrees warmer than 18°C. However, whereas the streams showed in May a maximum only a little below the highest temperature reached at any time in the summer, the lake warmed up slowly to a maximum in late summer and did not reach 18°C till towards the end of June. Nymphs would have emerged by this time and presumably the eggs are more resistant and can withstand the higher temperatures of late summer in the lake.

The life history of *Baëtis rhodani*, studied by both Pleskot (1958) and by myself (Macan 1957) illustrates a point of particular importance to a gathering of this kind — the value of doing the same thing in two different places. It also illustrates certain difficulties connected with work on streams, notably that it is not easy to find out the exact temperature conditions under which an animal develops, because the temperature is not the same at any two points and the animals move up or down the stream at different times of year.

I obtained some data about this movement by means of emergence traps. There were six stations altogether in the stream which I studied (Ford Wood Beck) and,

for the last seven years, emergence traps at two of them: Outgate near the source and station 1 near the mouth. Nymphs of *Baëtis rhodani* were always more abundant at st. 1 than at Outgate, though there were always fair numbers at the latter station. Emerging adults, on the other hand, were rare in Outgate; in 1955, a year of unusual abundance of all species, 20 were taken but in the other six years the total catch was 4. There have always been plenty in the trap at st. 1, from which it is evident that most nymphs move downstream before emergence.

The temperature at Outgate was measured by means of a thermograph over a period of years, and the resulting wealth of data makes one realize how difficult it is either to characterize the temperature in a few words or to make too close a comparison with streams in which readings were taken only occasionally. Pleskot describes the temperature of my stream as "maximum meist 16 bis 17° C", which is as fair a summary as anyone could make in five words. Table 1 shows in greater detail the figures on which this summary is based. St. 1 is 2—3° C warmer than Outgate (Macan 1958). Throughout most of 1959 the maximum lay between 19 and 20.5° C though in August it reached 22° C. A maximum of 18° C was recorded in Outgate that year, though not with the thermograph, which had been removed. Pleskot records "maximum über 20° C" in her stream. The interesting difference between the two is that, whereas in mine a quick summer generation of *B. rhodani* has started to emerge before the over-wintering generation has finished, in hers there is a gap of a month and a half between the two during late summer. This is attributed to unfavourably high temperature. We are obviously reaching a stage when only laboratory experiments can establish exactly the critical temperatures which are beginning to become apparent from this field work, but I believe that these field results are an indispensable preliminary to indoor investigations.

Table 1

The figures show the number of hours during which the temperature was between the levels indicated.

	1952	1954	1955	1956
above 19° C . .	3	0	0	0
18—19° C . . .	12	0	1	0
17—18° C . . .	40	0	82	0
16—17° C . . .	165	7	342	0

Harker (1953) and Macan (1957) have written accounts of the Ephemeroptera of small stony streams. Six species, *Rhithrogena semicolorata*, *Ecdyonurus torrentis* or *venosus*, *Heptagenia lateralis*, *Baëtis rhodani*, *B. pumilus*, and *Ephemerella ignita* appear to be characteristic of such a biotope, not only in Britain but on the continent too, as the thorough surveys of Illies (1952) and Dittmar (1955) show. Both authors record a few species that do not occur in Britain. The list becomes longer as current slackens and as size increases. Recent changes in the fauna of Ford Wood Beck are probably correlated with the bringing of water to the village of Outgate and the consequent over-loading of its one septic tank, which overflows into the stream. *Polycelis felina* (*cornuta*) has become much more numerous (table 2), and *Simulium* spp. and various net-spinning Trichoptera have also increased. *Ecdyonurus torrentis* disappeared from the emergence trap catches in 1959, and both *Baëtis rhodani* and *Rhithrogena semicolorata* have been sinking steadily since a peak in 1955; the latter, the most abundant species in 1953, was not taken in 1960. The wild fluctuations of *Baëtis pumilus* are inexplicable and no trend can be made out. *Ephemerella ignita* appears unaffected though collections in the

water indicate that it is more widespread than formerly. I suggest that the pollution did not affect the fauna through a toxin, because that should have caused an earlier and more abrupt decline and disappearance. Nearly all the houses were connected to the water supply by 1953 and the high numbers of so many species (Plecoptera were relatively more abundant than Ephemeroptera) in 1955 (table 2) suggests that these species were favoured by the pollution. Their subsequent decline must have been due to the activities of some other animal which could exploit the situation, presumably an increased food supply of organic matter, bacteria, fungi and ciliates, more efficiently. Such an animal is *Polycelis felina* (*cornuta*). Jennings (1957) describes how it traps animals in mucus nets and can capture quite large specimens. If the small flatworms have been enabled to survive in large numbers by food originating in the septic tank, the resulting adult population could well have produced the observed fall in the numbers of certain Ephemeroptera. I have made no direct observations, but the fact that the species that move over stones have suffered more severely than those such as *Baëtis pumilus* and the Plecoptera which live in the gravel, supports the hypothesis.

Table 2

Catches in Ford Wood Beck. The first five species were taken in an emergence trap near the mouth, *Habrophlebia fusca* at Outgate near the source. The totals for *Polycelis felina* are those obtained with a net at five stations in March.

	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961
Baëtis rhodani	—	—	153	66	140	178	77	30	15	36	7	—
B. pumilus	—	—	86	7	3	8	103	127	7	4	50	—
Rhithrogena semicolorata	—	—	37	109	65	112	31	30	19	4	0	—
Ecdyonurus torrentis	—	—	8	10	6	22	5	16	1	0	0	—
Ephemerella ignita .	—	—	6	10	34	76	19	7	2	9	22	—
Habrophlebia fusca	—	—	—	—	0	11	81	79	17	48	109	—
Polycelis felina ...	2	2	6	—	—	—	52	—	435	1052	291	633

The only species to have increased is *Habrophlebia fusca*, which has become abundant at Outgate. Previously I had supposed that its scarcity in the beck was due to a current just faster than its optimum but these findings suggest that food supply rather than current is the determining factor.

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SYMPOSIUM XIII

PLECOPTERA

DIE UNTERORDNUNGEN, FAMILIEN UND GATTUNGEN DER PLECOPTERA

JOACHIM ILLIES, Deutschland

Im Katalog von Claassen (1940) wurde die erste moderne Zusammenstellung des Plecopteren-Systems gegeben. Eine entschiedene Verbesserung erfuhr dieses System durch die Darstellung von Ricker (1950), in welcher auch erstmalig die Verbreitungsgebiete der einzelnen Familien angegeben wurden. Seit jener Publikation sind, besonders aus der Südhemisphäre (Illies 1958ff.), zahlreiche neue Formen bekannt geworden, welche erhebliche Umstellungen und Erweiterungen des Plecopteren-Systems bedingten. Es ist daher notwendig, unsere gegenwärtige Kenntnis der Klassifikation der Ordnung Plecoptera in einer neuen Übersicht darzustellen.

Die folgende Liste der Unterordnungen und Familien enthält alle gültigen Gattungen mit ihrer Verbreitung. Die Ordnung gliedert sich danach in 3 Unterordnungen, 16 Familien, 174 Gattungen und ca. 1640 Arten. Im Claassen-Katalog von 1940 sind ca. 1340 Arten angeführt: trotz sehr zahlreicher Neubeschreibungen hat die Artenzahl also in den letzten 20 Jahren nur um ca. 300 zugenommen, ein Ausdruck dafür, daß die gründliche Revision vieler Gruppen zahlreiche alte Namen als Synonyme entlarvte. (Die Zahl der jemals für Plecopteren vergebenen Gattungsnamen — gültige und ungültige — liegt bei etwa 300!) Für die Perlidae ist eine gründliche Revision eine der wichtigsten Aufgaben der zukünftigen Plecopterologie: zahlreiche Arten und auch viele Gattungen (besonders die von Navás und einige von Klapálek) sind ungenügend begründet und werden vermutlich bei einer Revision der südamerikanischen und asiatischen Formen als Synonyme fallen. Andererseits ist damit zu rechnen, daß die meisten der von Ricker (1950, 1952) als Subgenera bezeichneten Gruppen zu echten Gattungen aufgewertet werden müssen. Bei Nemouridae, Taeniopterygidae und Leuctridae ist diese Aufwertung in der folgenden Liste bereits durchgeführt worden. Aber auch bei den Perlodidae (besonders in den Gattungen *Arcynopteryx* und *Isogenus*) und Perlidae (hier besonders Gattung *Acroneuria*) wird sicherlich ein Teil der jetzt als Subgenera geführten und daher in der folgenden Liste nicht enthaltenen Gruppen den Wert echter Gattungen besitzen.

Auch die von Ricker 1950 durchgeführten Degradierungen zu Subfamilien werden (in Übereinstimmung mit den meisten übrigen Autoren) für die Scopuridae, Nemouridae, Leuctridae, Capniidae und Taeniopterygidae wieder aufgehoben: alle diese Gruppen sind nach unserer Ansicht echte Familien. Eine ausführliche Begründung für die Umstellung der Notonemourinae von den Nemouridae zu den Capniidae wird von mir a. a. O. gegeben werden. Das Gleiche gilt für die Ersetzung des Familiennamens Abranchioperlidae Illies durch Senzillidae Navás.

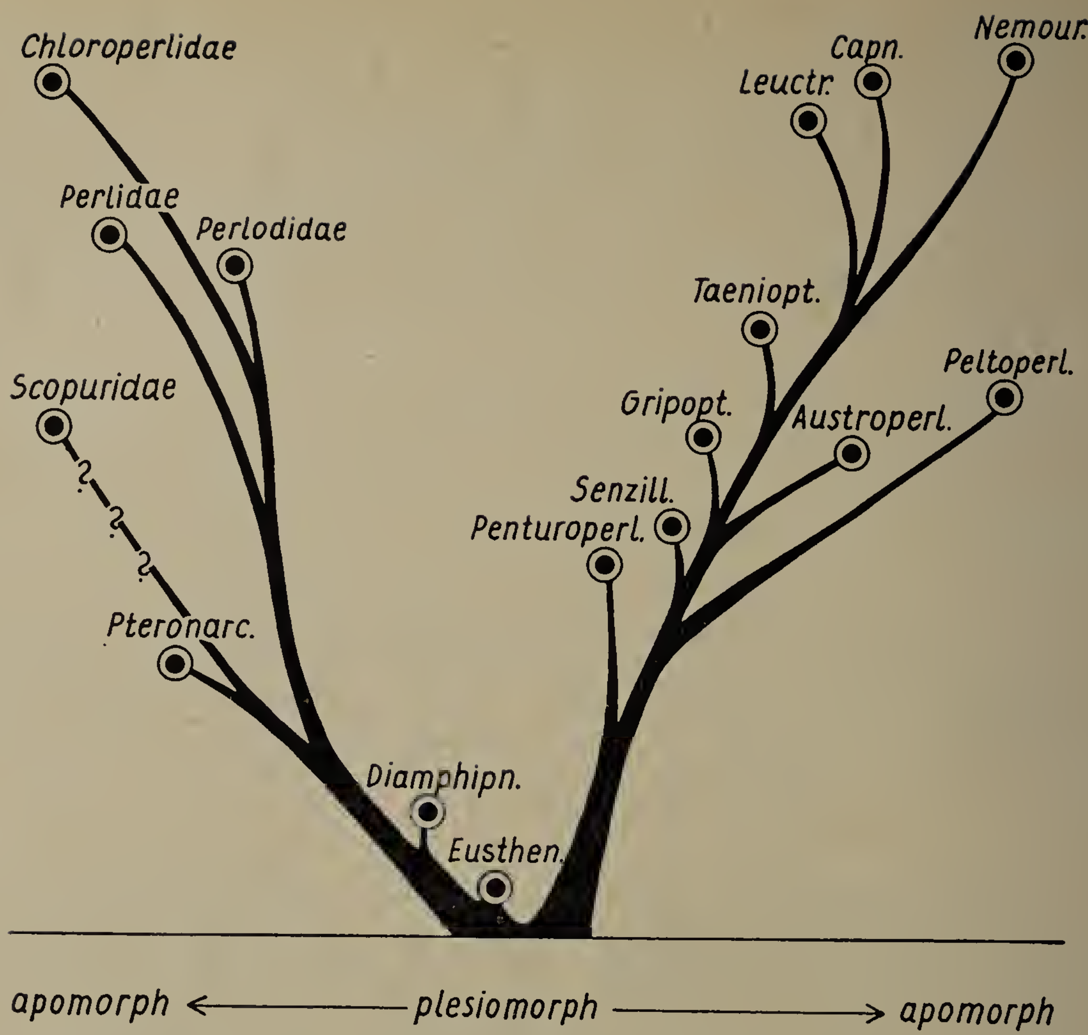

Abb. 1. Die Familien der Plecopteren in ihren phylogenetischen Beziehungen (nach Illies 1961).

Die Anordnung der Unterordnungen, Familien und Subfamilien in der folgenden Aufstellung entspricht ihren mutmaßlichen phylogenetischen Beziehungen und folgt der evolutiven Linie von den jeweils primitivsten (plesiomorphen) zu den abgeleiteten (apomorphen) Formen. Die anschließende Aufzählung der Gattungen geschieht in alphabetischer Reihenfolge.

I. Subordo Archiperlaria Illies 1960

1. Familie *Eustheniidae* Tillyard 1921.

Subfam. Stenoperlinae Tillyard 1921 (mit 3 Arten in Australien, Neuseeland und Chile verbreitet). *Stenoperla* McL. 1866 (Australien, Neuseeland), *Neuroperla* Illies 1960 (Chile).

Subfam. Eustheniinae Tillyard 1921 (mit 11 Arten in Australien und Chile verbreitet). *Eusthenia* Westwd. 1832 (Tasmanien, Australien), *Eustheniopsis* Till. 1921 (Tasmanien, Australien), *Neuroperlopsis* Illies 1960 (Chile).

Subfam. Thaumatoperlinae Tillyard 1921 (mit 3 Arten im südöstl. Australien verbreitet). *Thaumatoperla* Till. 1921 (Australien).

2. Familie *Diamphipnoidae* Ricker 1950 (mit 5 Arten in Chile verbreitet). *Diamphipnoa* Gerst. 1873 (Chile), *Diamphipnopsis* Illies 1960 (Chile).

II. Subordo Filipalpia Klapálek 1905

1. Familie *Penturoperlidae* Illies 1960 (mit 6 Arten in Australien und Chile verbreitet). (?) *Eunotoperla* Till. 1924 (Australien), *Klapopteryx* Navás 1928 (Chile), *Penturoperla* (Chile) Illies 1960.

2. Familie *Senzillidae* Navás 1917 (mit ca. 5 Arten in der westl. Neotropis verbreitet). *Notoperla* Enderl. (= *Senzilla* Navás = *Abranchioperla* Illies) (Argentinien, Chile).

3. Familie *Austroperlidae* Tillyard 1921 (mit 4 Arten in der Australis verbeitet). *Australoperla* Needh. 1905 (Neuseeland), *Tasmanoperla* Till. 1921 (Australien, Tasmanien).

4. Familie *Gripopterygidae* Enderlein 1909, Subfam. Gripopteryginae Enderlein 1909 (mit ca. 75 Arten in Australis und Neotropis verbreitet). *Antarctoperla* Enderl. 1905 (Feuerland), *Apteryoperla* Wisely 1953 (Neuseeland), *Aucklandobius* Enderl. 1909 (Auckland-Inseln), *Dinotoperla* Till. 1921 (Australien, Tasmanien, Neuseeland), *Gripopteryx* Pict. 1841 (Neotropis), *Leptoperla* Newm. 1839 (Australien, Fidji-Inseln), *Megaleptoperla* Till. 1923 (Neuseeland), *Nesoperla* Till. 1923 (Neuseeland) *Trinotoperla* Till. 1924 (Australien), *Zelandobius* Till. 1921 (Neuseeland), *Zelandoperla* Till. 1923 (Neuseeland).

Subfam. Andiperlinae Aubert 1956 (mit 2 Arten in Patagonien verbreitet). *Andiperla* Aubert 1956 (Argentinien), *Megandiperla* Illies 1960 (Chile).

5. Familie *Peltoperlidae* Claassen 1931 (mit ca. 30 Arten in östl. Paläarktis und Nearktis verbr.). *Cryptoperla* Needh. 1909 (Indien), *Microperla* Chu. 1928 (Ostasien), *Nogiperla* Okam. 1912 (Japan, Formosa), *Neopeltoperla* Kohno 1945 (China, Hinterindien), *Peltoperla* Needh. 1905 (Ostasien, Nearktis).

6. Familie *Taeniopterygidae* Klapálek 1905 (mit ca. 60 Arten in der Holarktis verbreitet). (?) *Allonuria* Clssn. 1936 (Kamtschatka), *Brachyptera* Newp. 1851 (Europa, Nordafrika), *Doddsia* Needh.-Clssn. 1925 (Nearktis), *Kyphopteryx* Kimm. 1946 (Himalaya), *Obipteryx* Okam. 1922 (Japan), *Oemopteryx* Klap. 1902 (Holarktis), *Rhabdiopteryx* Klap. 1902 (Paläarktis), *Strophopteryx* Frison 1929 (Nearktis), *Taenionema* Banks 1905 (westl. Nearktis), *Taeniopteryx* Pictet 1841 (Holarktis).

7. Familie *Leuctridae* Klapálek 1905 (mit ca. 140 Arten in der Holarktis verbreitet). *Despaxia* Ricker 1943 (Nearktis), *Leuctra* Steph. 1835 (Holarktis), *Megaleuctra* Neave 1934 (Nearktis), *Moselia* Ricker 1943 (Nearktis), *Pachyleuctra* Despax 1929 (Europa), *Paraleuctra* Hanson 1941 (Nearktis), *Perlomyia* Banks 1906 (Nearktis), *Rhopalopsole* Klap. 1912 (Japan, Formosa), *Tyrrhenoleuctra* Consgl. 1957 (Europa, Nordafrika).

8. Familie *Capniidae* Klapálek 1905.

Subfam. Capniinae Klapálek 1905 (mit ca. 140 Arten in der Holarktis verbreitet). *Allocapnia* Clssn. 1928 (Nearktis), *Allocapniella* Kawai 1955 (Japan), *Apteroperla* Matsum. 1931 (Japan), *Capnia* Pictet 1841 (Holarktis), *Capnioneura* Ris 1905 (Europa), *Capnopsis* Morton 1896 (Europa), *Eocapnia* Kawai 1955 (Japan), *Eucapnopsis* Okam. 1922 (Japan, Nearktis), *Isocapnia* Banks 1938 (Japan, Nearktis), *Nemocapnia* Banks 1938 (Japan, Nearktis), *Neocapniella* Clssn. 1936 (Sibirien), *Paracapnia* Hanson 1946 (östl. Nearktis), *Takagripopteryx* Okam. 1922 (Japan).

Subfam. Notonemourinae Ricker 1950 (mit ca. 40 Arten an der Südspitze aller südhemisphärischen Kontinente und benachbarten Inseln verbreitet). *Aphanicerca* Till. 1931 (Südafrika), *Aphanicercella* Till. 1931 (Südafrika), *Aphanicercopsis* Barnd. 1934 (Südafrika), *Austronemoura* Aubert 1960 (Chile), *Desmonemoura* Till. 1931 (Südafrika), *Kimminsoperla* Illies 1961 (Tasmanien), *Madenemura* Pauln. 1949 (Madagaskar), *Neofulla* Clssn. 1936 (Chile), *Neonemura* Navás 1919 (Chile), *Notonemura* Till. 1923 (Neuseeland), *Spaniocerca* Till. 1923 (Australien, Tasmanien, Neuseeland), *Spaniocercoides* Till. 1938 (Neuseeland), *Tsaranemura* Pauln. 1949 (Madagaskar), *Udamocercia* Enderl. 1909 (Chile).

9. Familie *Nemouridae* Klapálek 1905 (mit ca. 250 Arten in der Holarktis verbreitet, einzelne Arten in der orientalischen Region). *Amphinemura* Ris 1902 (Holarktis, Sunda), *Lednia* Ricker 1952 (westl. Nearktis), *Malenka* Ricker 1952 (westl. Nearktis), *Nemoura* Pictet 1841 (Holarktis), *Nemurella* Kempny 1898 (Europa), *Ostrocerca* Ricker 1952 (Nearktis), *Paranemura* Needh.-Clssn. 1925 (östl. Nearktis), *Podmosta* Ricker 1952

(Nearktis), *Prostoia* Ricker 1952 (Nearktis), *Protonemura* Kempny 1898 (Paläarktis), *Shipsa* Ricker 1952 (Nearktis), *Soyedina* Ricker 1952 (Nearktis), *Visoka* Ricker 1952 (westl. Nearktis), *Zapada* Ricker 1952 (Nearktis).

III. Subordo Subulipalpia (= Setipalpia) Klapálek 1905

1. Familie *Pteronarcidae* Enderlein 1909 (mit 16 Arten in der Nearktis und östl. Paläarktis verbr.). *Pteronarcella* Banks 1900 (Nearktis), *Pteronarcys* Newm. 1838 (Nearktis, östl. Paläarktis).

2. Familie *Scopuridae* Uéno 1938 (nur eine Art in Japan und Korea). *Scopura* Uéno 1929 (Japan, Korea).

3. Familie *Perlodidae* Klapálek 1912.

Subfam. Isogeninae Ricker 1943 (mit ca. 80 Arten in der Holarktis verbreitet). *Arcynopteryx* Klap. 1904 (Holarktis), *Hedinia* Navás 1936 (China), *Isogenus* Newm. 1833 (Holarktis), *Oroperla* Needh. 1933 (Kalifornien), *Pseudomegarcys* Kohno 1946 (Japan), *Suzukia* Okam. 1912 (Japan).

Subfam. Perlodinae Klapálek 1912 (mit ca. 30 Arten in der Paläarktis und westl. Nearktis verbr.). *Diura* Billbg. 1820 (westl. Nearktis, Paläarktis), *Perlodes* Banks 1903 (Paläarktis).

Subfam. Isoperlinae Frison 1942 (mit ca. 100 Arten in der Holarktis verbreitet). *Calliperla* Banks 1948 (westl. Nearktis), *Isoperla* Banks 1906 (Holarktis), *Nanoperla* Banks 1947 (Ohio), *Perliola* Banks 1947 (Colorado), *Rickeria* Jewett 1954 (Oregon), *Walshiola* Banks 1947 (Michigan).

4. Familie *Perlidae* McLachlan 1886.

Subfam. Perlinae McLachlan 1886 (mit ca. 360 Arten in der Paläarktis und Orientalis verbreitet, wenige Arten in der östl. Nearktis und 1 in der Aethiopis). *Agnetina* Klap. 1907 (Asien), *Cerconychia* Klap. 1913 (Formosa), *Dinocras* Klap. 1907 (Europa, Sibirien), *Dyaperla* Banks 1939 (Borneo), *Eoperla* Illies 1956 (Südeuropa, Nordafrika), *Esera* Navás 1909 (Kaukasus), *Etrocorema* Klap. 1909 (Hinterindien), *Formosita* Klap. 1914 (Ostasien, Sunda), *Harrisiola* Banks 1948 (westl. Nearktis), *Hemimelaena* Klap. 1907 (Spanien, Nordafrika), *Kamimuria* Klap. 1907 (Ostasien), *Marthamea* Klap. 1907 (Paläarktis), *Nakaharia* Navás 1916 (Japan), *Neoperla* Needh. 1905 (Orientalis, Aethiopis, Holarktis), *Neoperlops* Banks 1939 (China, Ceylon), *Neophasgonophora* Lestg. 1922 (westl. Nearktis, Ostasien), *Occiperla* Banks 1947 (westl. Nearktis), *Oodeia* Klap. 1921 (Hinterindien), *Oyamia* Klap. 1907 (östl. Paläarktis), *Paragnetina* Klap. 1907 (westl. Nearktis, Ostasien), *Perliphanes* Banks 1947 (New Mexico), *Perla* Geoffr. 1764 (Paläarktis), *Phanoperla* Banks 1938 (Philippinen), *Styloperla* Wu 1935 (China), *Tetropina* Klap. 1909 (Orientalis), *Togoperla* Klap. 1907 (Ostasien), *Tylopyge* Klap. 1913 (Ostasien).

Subfam. Acroneuriinae Klapálek 1914 (mit ca. 180 Arten in der östl. Paläarktis, Nearktis und Neotropis verbreitet). *Acroneuria* Pictet 1841 (Ostasien, Nearktis), *Anacroneuria* Klap. 1909 (Nearktis, Neotropis), *Atoperla* Banks (China, Nearktis), *Brahmana* Klap. 1914 (Indien), *Caroperla* Kohno 1946 (Japan), *Claassenia* Wu 1934 (östl. Paläarktis, Nearktis), *Coeloperla* Navás 1936 (Brasilien), *Collampla* Navás 1929 (Brasilien), *Diperla* Navas 1936 (Brasilien), *Enderleina* Jewett 1960 (Brasilien), *Eutactophlebia* Klap. 1914 (Brasilien), *Folga* Navás 1918 (Philippinen), *Gibosia* Okam. 1912 (Indien, Ostasien), *Inconeura* Klap. 1916 (Peru), *Kalidasia* Klap. 1914 (Hinterindien), *Kempnyia* Klap. 1914 (Brasilien), *Kiotina* Klap. 1907 (Ostasien), *Klapalekia* Clssn. 1936 (Kolumbien), *Laeissa* Navás 1934 (Brasilien), *Macrogynoplax* Enderl. 1909 (Neotropis), *Mesoperla* Klap. 1913 (Formosa), *Mesoperlina* Klap. 1921 (östl. Paläarktis), *Neoeuryplax*

Clssn. 1936 (Hinterindien), *Niponiella* Klap. 1907 (Japan), *Nirvania* Klap. 1914 (China), *Onychoplax* Klap. 1914 (Brasilien), *Perlesta* Banks 1906 (Nearktis), *Perlinella* Banks 1900 (Nearktis), *Schistoperla* Banks 1937 (Formosa), *Sinoperla* Wu 1948 (China).

5. Familie *Chloroperlidae* Okamoto 1912.

Subfam. Chloroperlinae Okamoto 1912 (mit ca. 90 Arten in der Holarktis verbreitet). *Alloperla* Banks 1906 (Nearktis, Japan), *Chloroperla* Newm. 1836 (Paläarktis), *Haploperla* Navás 1934 (Ostasien), *Hastaperla* Ricker 1935 (Nearktis), *Isoptena* Enderl. 1909 (Europa).

Subfam. Paraperlinae Ricker 1950 (mit 3 Arten in der westl. Nearktis verbreitet). *Kathroperla* Banks 1920 (westl. Nearktis), *Paraperla* Banks 1906 (westl. Nearktis), *Utaperla* Ricker 1952 (westl. Nearktis).

BEGATTUNGSORGANE UND SPERMAÜBERTRAGUNG BEI DEN ISOGENINEN PERLODIDEN

PER BRINCK

In einer Arbeit 1956 (Reproductive system and mating in Plecoptera, Opusc. Ent.) beschrieb ich die Genitalien einer Reihe von europäischen Perlodiden. Davon gehörte eine Art der Unterfamilie Isogeninae an, die vor allem durch das gespaltene 10. Tergum charakterisiert ist. Zwischen den beiden Hemitergiten liegt ein sog. Supraanallobus. Bei der Bildung dieses Lobus, in der Tat ein eigenartig ausgeformtes Organ, wirkt das 11. Tergum (Epiproct) mit.

Voriges Jahr war es mir während eines Besuches in den inneren Fjeldgebieten Schwedisch-Lapplands möglich, weitere Studien über Bau und Funktion der Begattungsorgane einer der interessantesten Arten unter den Isogeninen Perlodiden, nämlich *Arcynopteryx compacta*, vorzunehmen.

Im Hinterleibsende dieser Art finden sich ein dorsales und ein ventrales Organ, die im Zusammenhang mit der Begattung funktionieren. Die Funktion des dorsalen Organs war bisher unbekannt. Das ventrale Organ ist das Kopulationsorgan (Aedeagus), das schon früher von mir beschrieben worden ist. Es ist eine membranöse Bildung, die in Ruhe in einer Tasche versteckt ist.

Das dorsale Organ besteht aus einem Sack, der in Ruhe zwischen zwei kräftigen lateralen Laminae eingestülpt ist. Diese Balken sind apikal hakenförmig umgebogen und hier festigen sehr kräftige Längsmuskeln, die vorn an zwei Stellen inserieren: teils am Vorderrand des 10. Tergums, teils und hauptsächlich an der Basis einer Ankerplatte, die sich am Vorderrand des 9. Tergums durch zwei Schenkel stützt. Diese Schenkel sind am inneren, hinteren Rand der beiden Hemitergiten befestigt. Die Ankerplatte ist ventral herausgezogen und bildet hier ein bogenförmiges Stück, an dessen Spitze eine lange, gebogene Borste durch eine elastische Gelenkplatte befestigt ist. Die innere Fläche des Sackes ist verhältnismäßig eben, ein Paar Flecke an der Seite des Anfestungspunktes des peitschenförmigen Apikalanhanges ausgenommen. Diese Flecke sind mit dichten Dornfeldern bewaffnet.

Bei der Funktion des dorsalen Organes tritt der peitschenförmige Anhang aus dem Sack heraus. Dies wird dadurch hervorgebracht, daß sich die kräftigen Längsmuskeln entlang den Balken auf den beiden Seiten des Sackes kontrahieren. Sie wirken dabei als Lävator: die Ankerplatte wird hinten und aufwärts gezogen und dabei wird das

bogenförmige Stück mit dem peitschenförmigen Endanhang erhoben. Da der Boden des Sackes am bogenförmigen Sklerit befestigt ist, wird der Sack zwischen den beiden Balken herausgestülpt. Er wird dabei umgekehrt, so daß die Innenseite zur Außenseite wird. Der peitschenförmige Anhang findet sich also an der Spitze des herausgestülpten Sackes anstatt an dessen Boden.

Das weibliche Organ besteht aus einer einfachen Genitalkavität am 8. Sternum, die von einer Subgenitalplatte bedeckt ist. Dorsal sitzt ein Receptaculum seminis, das durch einen ziemlich langen Receptaculargang mit der Genitalkavität verbunden ist. Die Einzelheiten wurden schon von mir (1956) beschrieben und es ist nicht nötig, dies hier wieder zu besprechen.

Es zeigte sich jetzt, daß die Anwendung dieses dorsalen Organes mit der Kopulation intim verbunden war, wie folgt.

Bei dieser Art findet man wie bei vielen anderen Plecopteren ein einfaches Paarungsspiel. Wenn das Männchen ein Weibchen sieht, läuft es schnell auf es zu und ergreift es gewaltig mit seinen Füßen, ganz unabhängig von der Stellung seiner Partnerin. Dann läuft er über ihren Körper, dreht sich um und kehrt nach der anderen Seite zurück. In einigen Fällen wurde dies mehrmals wiederholt. Wenn das Weibchen paarungsbereit war, blieb es still oder bewegte sich leicht, wenn nicht, lief es schnell davon. Wenn es paarungsbereit war, stellte sich das Männchen dann über seine Partnerin. Er preßte Kopf und Thorax gegen den weiblichen Vorderkörper und tastete und trommelte mit den Palpen an ihren Kopf. Sein Hinterleib war mit dem weiblichen Körper parallel und ruhte auf ihrem Hinterleib.

Einige Minuten nach dem Anfang des Spiels legte er seinen Hinterleib an die Seite des weiblichen Abdomens, krümmte ihn und versuchte die Spitze unter die Hinterleibspitze des Weibchens einzuführen. Jetzt trat das dorsale männliche Organ in Funktion. Es war leicht zu beobachten, wie es teilweise herausgestülpt wurde, anschwoll und zu pulsieren begann, so daß der sklerotisierte, peitschenförmige Anhang hinaus und hinein geschoben wurde. Das Männchen preßte so seine Hinterleibspitze unter die des Weibchens und die knospenförmigen Teile der Hemitergiten des 10. Segmentes wurden gegen den Hinterrand der weiblichen Subgenitalplatte angelegt und teilweise zwischen den Rand und das Sternum eingepreßt, so daß die Vulva geöffnet wurde. Dann wurde das ganze dorsale Organ herausgestülpt und in die Vulva hineingeführt. Das dorsale Organ führte jetzt kräftige pulsierende Bewegungen, wie auch kräftige Drehungen seitwärts während ungefähr einer Minute aus.

Es gelang mir, bei mehreren Pärchen festzustellen, daß das ventrale männliche Organ (Aedeagus) an diesen Bewegungen nicht beteiligt war: es konnte überhaupt nicht gesehen werden.

Nach einer Minute wurde das ventrale Kopulationsorgan des Männchens plötzlich herausgestülpt und sehr schnell über das dorsale Organ in die Vulva hineingeführt. Dabei zog sich das dorsale Organ unmittelbar zurück. Wenige Sekunden später folgte die Übertragung der Spermatophore und bald danach wurde das männliche Kopulationsorgan herausgezogen und das Pärchen trennte sich.

Bei dieser Art lassen sich also bei der Spermaübertragung 4 verschiedene Momente feststellen:

1. Einleitendes Spiel.
2. Einführen des dorsalen Organes in die weibliche Vulva, was von kräftigen Bewegungen des Organes begleitet wird.
3. Einführen des ventralen Kopulationsorganes (Aedeagus) und Deponierung der Spermatophore.
4. Trennung des Pärchens.

Die Momente 1., 3. und 4. stimmen mit den gleichen Vorgängen bei anderen Plecopteren überein. Aber wozu dient eigentlich das dorsale Organ, das oberflächlich gesehen ungefähr so verwendet wird wie ein echtes Kopulationsorgan? Die älteren Autoren sagten, daß so ein Organ ein Titillator sei. An und für sich wäre wohl diese etwas anthropomorphe Deutung möglich, aber ich selbst möchte dies nicht als den hauptsächlichen Zweck des Organes ansehen.

Nach vielen vergeblichen Versuchen gelang es mir, ein Pärchen so in Copula zu fixieren, daß man den peitschenförmigen Anhang verfolgen konnte. Es zeigte sich dabei, daß die weichen Teile des Organes die Genitalkavität des Weibchens füllten, während der Anhang den langen, mit einer sklerotisierten Intima versehenen Ductus receptaculi teilweise penetrierte. Es ist wohl also möglich, daß wir es hier mit einem Spezialorgan zu tun haben, das die Öffnung des Ductus receptaculi besorgt, so daß die unmittelbar nach der Reinigung des Ganges eindringenden Spermatozoen ohne Schwierigkeiten nach dem Receptaculum seminis vordringen können.

ON THE MUSCLES OF THE REPRODUCTIVE APPARATUS OF ISOPERLA GRAMMATICA (Poda)

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In collaboration with Prof. Per Brinck, the author has studied the reproductive organs of *Isoperla grammatica* (Poda, 1761), especially as regards its musculature (Brinck & Froehlich, 1960). The reproductive organs of the species under consideration, together with that of several other Plecoptera, are dealt with in a more general way in Brinck, 1955. Here a brief survey of the genital musculature is presented.

Male. The male copulatory organ is a vesicle which is in rest completely concealed in the enlarged, ventral part of sternum IX, and which during mating is extroverted by pressure from the body fluids.

The copulatory organ is provided with numerous intrinsic muscle fibres, most of which originate at the penial armature. The strongest set of fibres originates at the ventral part of the armature, and the fibres, of various lengths, run ventral and lateral along the surface. The other important set originates at the sides of the armature and runs forward, usually as a pair of well-defined bundles which fan out anteriorly. The most important function of the intrinsic muscles is probably the ejaculation of sperm, brought about by reducing the volume of the copulatory organ.

From the body, a pair of strong retractors enter into the copulatory organ to fan out dorsally. These retractors originate from the anterior margin of sternum IX and are modified internal sternal muscles.

Also from the body, some loose fibres enter into the copulatory organ along its ventral surface. These fibres are part of a pair of muscles which originate at the sides of segment IX. Other fibres run to the fold which is formed around the peduncle of the copulatory organ, and still others to the ventral surface of the peduncle, some insertions being contralateral. These muscles seem to be modified lateral muscles.

During the first stages of protrusion these muscles, by dilating the passage through which the copulatory organ everts, aid in the process. They are also important in the retraction process, first by pulling in the proximal part of the copulatory organ, then by keeping open the passage for the rest to pass.

From the posterior part of sternum IX originate a pair of muscles, the fibres of which run to the dorsal part of the fold around the peduncle or, chiefly, to the dorsal surface of the latter. Most of the fibres are inserted contralaterally. As judged by their origin, these muscles are modified external sternal muscles. During protrusion, their contraction act as a clamp, pressing the dorsal wall of the peduncle downward, thus preventing the fluids within the copulatory organ to return to the body. This clamping action is probably very important during the ejaculation process. They aid also in the later stages of retraction, by pulling backward, into the extended portion of sternum IX, the proximal part of the copulatory organ.

The muscle coat of the male efferent system is weak, but a little stronger around the ejaculatory duct.

Female. The paired oviducts join to a very short common oviduct, directed backward and upward, which projects into the anterior, muscular part of the genital cavity as an elongated ridge. The gonopore is a slit along the edge of the ridge. The ridge is surrounded by a muscular, horseshoe-shaped dorsal flap, the anterior ends of which are fused to the wall of the cavity. The ridge and the flap form a sluicing apparatus. In the central hollow of the horseshoe is located the opening of the receptacular duct. The latter is moderately long and ends in a receptaculum seminis provided with accessory glands. The posterior part of the genital cavity is wide and flat, with membranous walls. It opens by a broad genital aperture above the subgenital plate.

The oviducts are surrounded by a muscle coat comprising both circular and longitudinal fibres. The common oviduct is surrounded by a strong muscle felt.

The pair of mesial bundles of the internal sternal muscles of segment VII converge to and insert at the anterior wall of the genital cavity. From around the insertion points of these muscles numerous fibres originate. Part of them radiate around the walls of the genital cavity, part run into the muscular flap. All these fibres are more or less longitudinal.

The mesial fibres of the internal sternal muscles of segment VIII also serve the genital cavity. They run around the lateral walls of the muscular part of the cavity and fan out posteriorly, some crossing behind the receptacular duct, some inserting on the surface of the posterior, non-muscular part of the cavity.

The receptacular duct has a strong coat of circular muscle fibres. The receptaculum, on the other hand, is almost devoid of a muscle coat. Strong longitudinal fibres surround the ducts of the accessory glands.

The sluicing apparatus is, as can be inferred from its structure, a device for insuring fertilization of the eggs. An egg, issuing from the gonopore, is retained by the dorsal flap and rests below the opening of the receptacular duct. After fertilization, contraction of the various muscles pull the flap forward, thus liberating the egg. At the same time the contractions reduce the volume of the anterior part of the cavity and push the egg backward via the membranous part of the cavity to the genital aperture.

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VISIBLE CHANGES PRECEDING MOLTING IN PLECOPTERA

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ABSTRACT

Ecdyses provide definite reference points in work on insect biology. Unfortunately, ecdysis s usually so rapidly accomplished that it is not observed. Therefore, indirect methods of determining whether or not a molt has occurred, such as exuvial remains and head capsule size changes, are used. These are very time-consuming efforts often subject to error.

In Plecoptera certain physiological activities preparatory to molting have been discovered ito produce visible evidences of an impending molt many days in advance. The disappearance of these features gives positive evidence that the insect under study has molted into a new instar since last observed.

The features which may be used as indicators of molting, roughly in order of appearance, are as follows: (1) an apparent shrinkage of the head of the newly forming instar within the exoskeleton, most conspicuously visible in the compound eyes, (2) the occurrence of abnormally long frass pellets, (3) the complete clearing of the gut of food contents and feces, (4) the thickening of the wing pads in the latter part of the last instar, and (5) the blackening of the wing pads of the last instar. None of these indicators is universally applicable but one or another is generally available for service in any particular species.

THE HATCHING AND GROWTH OF THE NYMPHS OF SEVERAL SPECIES OF PLECOPTERA

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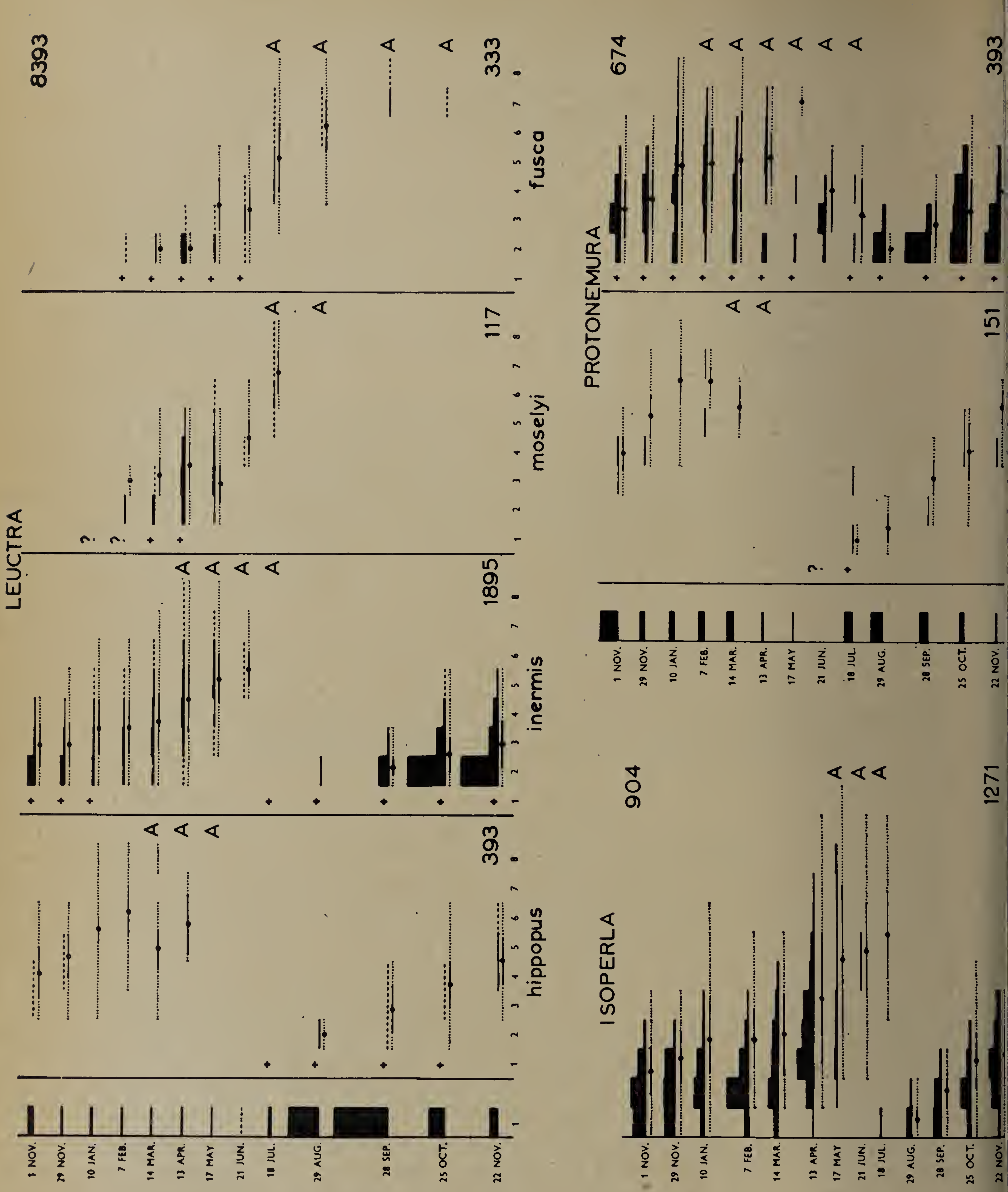
During an extensive study of the invertebrate fauna of a Welsh mountain stream (Hynes 1961) I obtained much information on the early stages of Plecoptera. It is reported upon here in much greater detail than has been possible previously.

Full details of the methods used are given in my earlier paper; it suffices here to state only that collections were made in a standardised manner, at approximately monthly intervals, from November 1955 to November 1956, and the same area of stream bed was sampled on each occasion. Nets of two mesh-sizes, 6 threads/cm. and 40 threads/cm. were used. It is with the latter that we are primarily concerned here, as this mesh is fine enough to retain even newly hatched stoneflies, but the data obtained from the coarse-mesh samples have been summarised in the figures as they serve at some points to clarify the picture given by the fine-mesh samples. The former did not, of course, include any very small specimens, but they gave, perhaps, a better indication of the upper limits of the size-range on some sampling dates.

All the specimens collected were identified and measured (from front of labrum to tip of abdomen) and were placed in size-groups: 1 = 0—1.5 mm. 2 = 1.5—2.5 mm. and so on. In most species, newly hatched nymphs, which are distinguishable by


LEUCTRA
hippopus
inermis
moselyi
fusca
393
1895
117
333
8393
PROTONEMURA
674
151
393
ISOPERLA
904
1271
1 NOV.
29 NOV.
10 JAN.
7 FEB.
14 MAR.
13 APR.
17 MAY
21 JUN.
18 JUL.
29 AUG.
28 SEP.
25 OCT.
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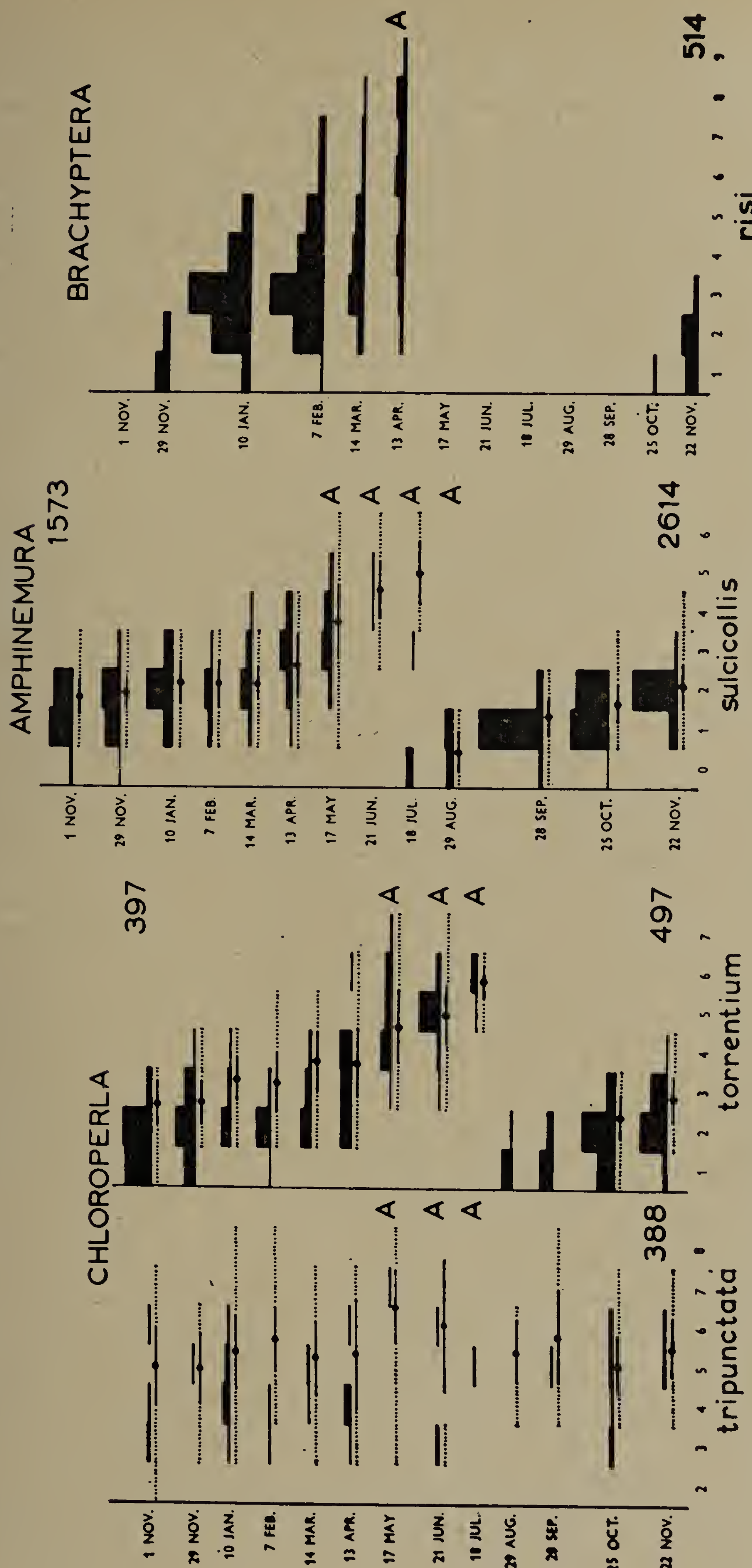

Fig. 1. The seasonal size distributions of Plecopteran nymphs from the Afon Hirnant. For further explanation see text.

having only few cercal segments, are about 0.5 to 1 mm. long, but those of *Amphinemura sulcicollis* are considerably smaller, so for this species a further size-group (0 = 0—0.5 mm.) was used. In those genera of which more than one species was present it was not always possible to be certain of the specific identity of every individual in the 1 mm. size-group, although it was usually possible to ascertain which species were represented in the size-group on each sampling date.

The stream, the Afon Hirnant in the county of Merionethshire, contains twenty species of Plecoptera, but some of these did not normally occur in the area sampled with the fine mesh, and others were too rare to be taken in reasonable numbers.

We are therefore concerned with only eleven species, the data for which are given in Figure 1. This has been constructed as follows. For each species or genus total numbers of specimens collected during the year by the fine mesh are shown top right. Histograms show the percentages of these totals, in size-groups, for each sampling date. A dashed line indicates less than 0.1%. Where, in *Leuctra* and *Protonemura*, there was doubt about the identity of the 1 mm. specimens, the percentages are shown separately in a column on the left, and small crosses in the 1 mm. column over the specific name indicate definite identification of some 1 mm. specimens. This was not done for *Chloroperla*, where I thought until recently that I had correctly identified all the very small specimens; it will be seen below, however, that I now have reason to doubt this.

The total number of individuals collected by the coarse net during the year is shown for each species at the bottom right. Below each monthly histogram is a line based on these samples. For each month the mean size is shown as a spot, the standard error of the mean as a continuous line, and the total size-range as a dotted line. For *Brachyptera risi* large specimens were not taken by the fine net, as they tend to live on large stones, inaccessible to the sampling apparatus; the histograms are therefore based on both fine and coarse-net samples added together. The symbol A on the right shows that adults were collected during the month.

These figures therefore show in detail the life histories, growth-rates, hatching-periods, emergence-periods, seasonal abundance and, within the genera containing two or more species, the relative abundance of the various species in a single stream. I think they illustrate many points of interest to students of the order. Some of these are as follows:

1. In many species, e. g. *L. hippopus*, *L. inermis*, *I. grammatica*, *P. meyeri* and *C. torrentium*, the period during which very small specimens can be found is much longer than the flight-period, indicating delayed hatching of some eggs, as has been observed in Ephemeroptera (Macan 1957, Illies 1959).

2. While this long hatching-period continues there is steady recruitment to the population such that the total number of individuals present does not fall much, despite inevitable losses from predation and other causes. Thereafter, however, the numbers present (i. e. the areas of the histograms) decline. This is well shown by the commoner species, i. e. *L. inermis*, *I. grammatica*, *C. torrentium* and *A. sulcicollis*, but is not shown by *P. meyeri*, in which recruitment seems to go on almost throughout the year.

3. In some species, e.g. *L. hippopus*, *P. praecox* and particularly *B. risi*, it appears that growth is active during the cold winter months. In others, e.g. *L. inermis*, *I. grammatica*, *C. torrentium* and *A. sulcicollis*, there is fairly clear evidence that growth slows down in winter time.

4. Late-hatched specimens seem to grow very fast, as compared with early-hatched ones, probably because they have the benefit of the warmer spring or early summer weather. This can be seen in the histograms for *L. inermis*, *I. grammatica* and *C. torrentium*. *B. risi* is a particularly interesting example of this because, although many specimens

were quite small in April, all had gone by the middle of May. Had they grown to maturity in this short time, or were the smaller ones, as seems to me more likely, unable to reach full size before being killed by rising temperature? This species seems to be a cold-water stenotherm, and it may be that some specimens are killed off by heat in all normal years.

5. Where more than one species of a genus is present, the species succeed one another in such a way that at any given time, even if as in *Protonemura* and *Chloroperla*, their flight-periods coincide, the average sizes of the nymphs are different. Thus *P. praecox nymphs* are always larger than those of *P. meyeri*, *L. hippopus* larger than *L. inermis* etc.

6. Despite this apparent "sharing out of niches" between closely allied species, which would seem to avoid competition between them, one species is in most instances considerably more abundant than the other, cf. *L. hippopus* and *L. inermis*, and *P. praecox* and *P. meyeri*. This is certainly a property of the habitat, as the reverse can occur elsewhere, but what causes it?

7. Finally a point of particular interest is the life history of *C. tripunctata*. *C. torrentium* clearly has a simple univoltine cycle, but no such simplicity is shown by the histograms for *C. tripunctata*. I obtained similar results many years ago and suggested then that it might take two years to develop (Hynes 1942). I then had doubts (Hynes 1961), but recent work on the Afon Hirnant has clarified the situation and enabled me to explain the figure given here. In 1959/60 intensive study is being made of one small area, and this has shown that newly hatched *C. torrentium* are not to be found after December. Very small *C. tripunctata*, however, were taken in some numbers in February and March, and some were 3 mm. long in early May, before the emergence of adults of the previous generation. In this species therefore, alone amongst the stoneflies, the eggs lie dormant for about nine months, and the nymphs require two summers for development. It appears that this interpretation will fit the rather scanty data in figure 1, if one assumes, as now seems likely, that two 1 mm. specimens (in February) were wrongly identified.

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SYMPOSIUM XIV

ODONATA

ON THE PROBLEM OF INTRINSIC AND ADAPTIVE CHARACTERS IN ODONATE LARVAE

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The only Odonata known to possess tracheated gill appendages along the sides of the abdomen, in addition to the usual three caudal abdominal gills, belong to the *Polythoridae* (neotropical) and *Epallagidae* (mainly oriental). These two families in current classifications are placed in the *Calopterygoidea* (= Agrionoidea). The paired gills are probably present in the mature larva of all members of the families just mentioned. In the Polythorid genera (*Cora* and allies) they occur on segments 2—7 as curled or twisted 1- to 3-segmented appendages while in the known Epallagid genera (*Anisopleura*, *Bayadera*, *Dysphaea*, *Epallage* and *Euphaea*) they are present on segments 2—8 as slightly undulated tapering unsegmented filaments. Up to the present only the full-grown larvae of these forms were known (cf. Calvert 1911, and Ris 1912).

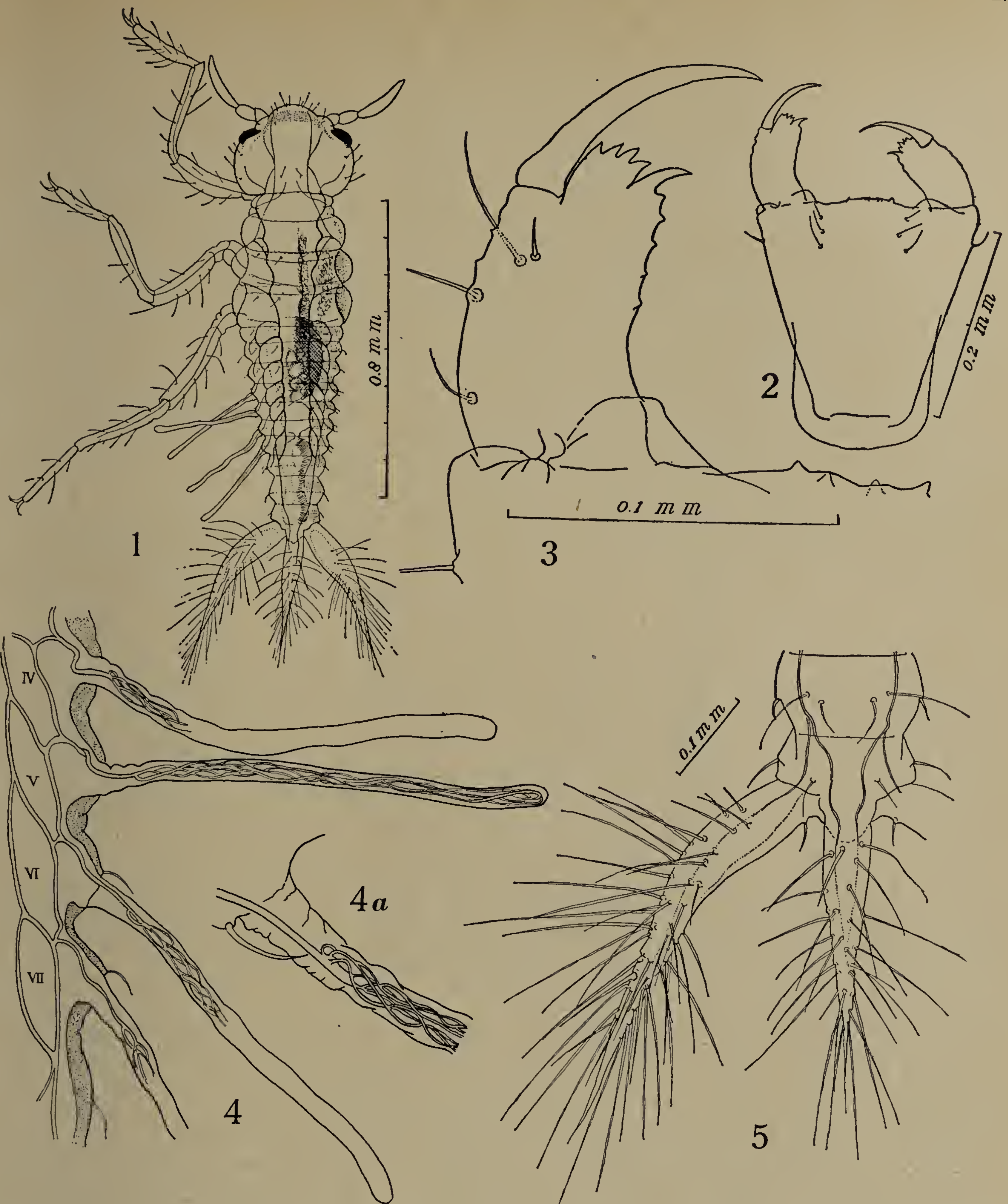
Late in December 1931, the newly hatched larva of *Euphaea variegata* was discovered in West Java. Eggs of this species (and of the Calopterygid *Vestalis luctuosa* as well) were obtained by collecting decaying vegetable matter in which the females had been observed ovipositing, all from the same jungle stream near Sukanegara, about 600 m above sea-level. Five eggs of *Euphaea* developed and hatched in confinement 15—16 days after being laid. The prolarva was not observed, but live specimens of the second instar were studied simultaneous with those of *Vestalis*. As all individuals died before the first moult it was impossible to follow up the changes through their different stages of growth.

It is from the earliest stages that the most reliable evidence is likely to be obtained as to the archetypic larva of Odonata. Amongst the chief peculiarities of structure exhibited by the very young *Euphaea* larva two characters deserve special attention, viz. the labium and the external abdominal gills. Both have followed their own lines of specialization during post-embryonic development.

The labium

In comparing the second instar labium of *Euphaea* and *Vestalis* (figs. 2—3 and 7—8), one is immediately impressed with their great similarity of structure. Its initial resemblance to that of the earliest stages of other Zygoptera and Aeshnoid dragonflies indicates that it has not been essentially modified in the larval evolution. Unfortunately, nothing appears to be known of the labial structure in newly hatched larvae of such archaic forms like *Hemiphlebia*, *Lestoidea* and those lying at the base of the Calopterygoidea (e.g. *Amphipteryx*), but even the labium of the second instar larva of the Anisozygoptera as figured by Asahina (1954) for *Epiophlebia*, shows that it is very similar and built according to the same plan.

The primitive form of labium exhibited by the Epallagidae and the Calopterygidae, of course, contrasts strongly with the divergence of their development in the same

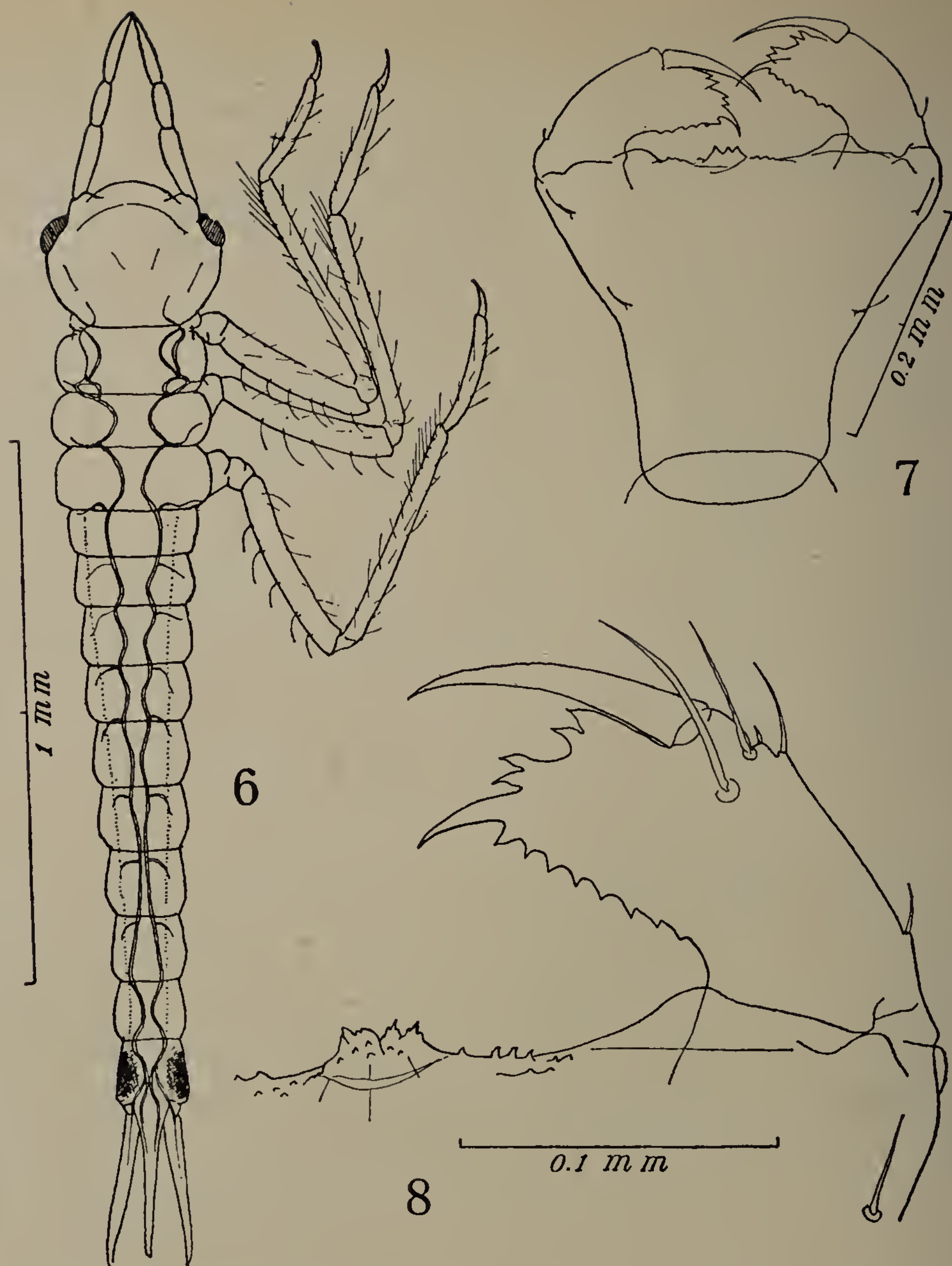


Euphaea variegata Ramb., from Sukanegara (West Java). Fig. 1, Newly hatched larva (live specimen), showing tracheal system and lateral gills (left side) and internal organs (right side only, slightly diagrammatic). Figs. 2—3, Interior view of labium and left lateral palpus, showing median lobe of prementum and setae. Figs. 4—4a, Left lateral abdominal gills, ventral view, showing ramifications of tracheal branches. Fig. 5, Apical abdominal segments and caudal gills.

groups during larval life. The amazingly various shapes in the fully grown stages are only too well known. They suggest independent specializations, and the structural changes they undergo at each moult are probably all adaptations to the environment.

External abdominal gills

In the newly hatched larva of *Euphaea* the caudal gills are remarkable only in being already in the form of a simple saccus. These are much slenderer than in the full grown larva, tapering to a point and furnished with numerous long setae. The



Vestalis luctuosa (Burm.), from Sukanegara (West Java). Fig. 6, Newly hatched larva (live specimen), showing main tracheal system. Figs. 7—8, Interior view of labium and right labial palpus, showing median lobe of prementum and setae.

arrangement of the main tracheae is quite typical: the bifurcation of the dorsal trunks occur in the 9th segment and there are two main tracheae in the median and one in the lateral saccus, but all of them are unbranched (figs. 1 and 5).

The paired lateral gills appear simultaneously on the intermediate segments 4-7 as transparent finger-shaped tubules. They are ventral in position, arising from the laterosternites of the segments and fall in line with the thoracic legs. Tracheation in them is richly developed, each gill containing a tracheal branch of the ventral trunk which soon ramificates to form a large number of capillaries. The recurrent tracheoles become connected with the twigs from which the efferent tracheoles arise so that the capillary circulation appears confined to the loops alone (fig. 4). This system is strongly reminiscent of that in the lamellae of the folds in the rectal gill-chamber of Anisopterid larvae (see, for instance, Oustalet 1869, pl. 24, fig. 13). Lateral branch tracheae of the ventral trunk similar to those occurring in the gill-bearing segments are already present and are undoubtedly tracheae *in statu nascendi* destined to penetrate the tubules of later instar larvae (fig. 1). Inferentially, their significance as respiratory organs would increase at the next ecdyses, i.e. during successive stages of growth. However, as development proceeds, they do not seem to increase in complexity and some kind of retrogression is likely to take place, possibly resulting in a slenderer form of gill which shows signs of becoming atrophied, because the full-grown larva has become an opaque and firmly built insect provided with filamentous tapered gills.

It is tempting to compare these tracheal structures with the respiratory organs of other insect larvae. Are the initial paired gills of *Euphaea* homologous with those of the ancestral Ephemeroptera, which are believed to have had similar simple tubular structures into which the tracheae enter? This must, indeed, remain an open question, since many larvae of admittedly archaic Zygoptera are entirely devoid of these structures.

Calvert (1928) examined the exuviae of second instar larvae of three different anisopterid species and discovered traces of initial spiracles as well as tracheal branchlets running to them; they were present in most (or all) of the abdominal segments 2—8. In the gill-bearing *Cora* larva these tiny spiracles even exist in the first segment, which is in accord with findings in the mature stage of Epallagid larvae. As in Ephemeroptera, these spiracles are still nonfunctional. The evidence from the early appearance of abdominal spiracles in both Ephemeroptera and Odonata corroborates previous conclusions that the two orders are derived from terrestrial ancestors which used these stigmata, and that implies air-breathing creatures. This conclusion, as Calvert cautiously adds, is independent of whether mayflies and dragonflies are at all related, and also whether or not Epallagidae and Polythoridae, with their paired external abdominal gills (in which respect they resemble the larvae of extinct as well as modern mayflies) are primitive within their own order.

Biological notes

Little or nothing is known of the habitat of Epallagid larvae and their behaviour under natural circumstances. Though not at all restricted in its rheophilous habitations, the *Euphaea* larva generally requires well aerated water and rocky streams for its development, but the very young and fully grown stages are probably both adapted to their own peculiar situation in the stream. Various aspects of the life history might be better understood through observations in the field and these are much needed to establish the relation between structural phenomena and habitat preference (see, for instance, Ide 1935, in Ephemeroptera, and Lieftinck 1950, in *Macromia*).

Owing to its transparency and minute size (ca. 1.5 mm), the newly hatched larva was never observed in samples collected in the stream. The creatures possibly cling to moss-bearing pieces of wood or hide themselves amongst the vegetation growing on rocks in which the adult oviposits. The projecting gills then might be of use to hold on to the substratum.

In captivity the new-born larva did not attempt to seek shelter under or on stones, but crawled about freely. Its lateral gills project sideways in a horizontal plane and can be moved slowly and independently to and fro in the water (fig. 1). Their respiratory function appeared evident. Rectal folds are present but their tracheation is unapparent; weak contractions and expansions of the rectal cavity were observed soon after the time of hatching. Respiration is probably continuous and effectuated through the integument and the two kinds of external gills combined.

The full-grown larva, on the other hand, was found either in midstream under stones in shallow water with a moderate current or amongst dead leaves accumulated between boulders, but never in rapid flowing water. Its body is deeply pigmented though not strongly sclerotized; the cuticle of the gills is relatively thin and the paired appendages are directed backwards, fitting closely to the sternal surface of the body when the insect is at rest. The respiratory function of the latter is possibly incidental, but published statements to the effect that they merely "act as anchoring devices to prevent them being swept away by the swift currents of the streams in which they live" (Fraser 1957), are merely conjectural. Larvae of *E. variegata* placed in a vessel clung to the substratum and kept their gills loosely folded together under the body. *Anisopleura*, according to the same author (loc. cit.) "is without these appendages but as they breed in slow water-

courses, such as irrigation-channels and sluggish streams, the need for them has not arisen". This deduction is premature, as Needham (1911) found them to be well developed in the larva of *Anisopleura comes*, and I established their presence in the allied genus *Dysphaea*. In exuviae the gill filaments are very easily overlooked and this has presumably been the case in the latter instance quoted.

The full-grown larva of *Epallage fatime* is almost exactly similar to that of *Euphaea*. It was found by Popova (1953) on stones in clayey sand-bottomed streams at some distance from the bank, at depths of 30—40 cm but with a strength of current varying from 0.37—1.32 m/sec. The sinusoid shape of the paired gills is supposed to have some significance as an adaptation to life in rapid running water as this form would admit the strong current to pass more smoothly under the body, thus protecting the fragile structure of the gills. In this connexion a remarkable observation recently published by the Swiss naturalist Robert (1958) is worth attention. He says of the same species (translated from French): "Their larvae live in streams that sometimes dry up as a result of (prolonged) heat. Accordingly they are forced to keep in shallow pools or even in overheated sand and need supplementary gills". Thus even the gill-bearing larvae of *Epallage* would be able to resist periodical droughts.

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UNTERSUCHUNGEN ZUM TERRITORIALEN VERHALTEN VON AESCHNA CYANEA MÜLL.

GERALD MAYER

St. Quentin spricht den Anisopteren generell ein Jagdrevier zu und definiert ein solches Revier durch Abgrenzung, Verteidigung und längeren Aufenthalt des Revierinhabers. Da Markierungen der Tiere, die klare Beweise für das Vorhandensein eines Revierbesitzers erbracht hätten, fehlschlugen, entfernte St. Quentin bei *Aeschna cyanea* jeweils am Abend den Revierinhaber und stellte fest, daß das entsprechende Revier am nächsten Morgen stets wieder besetzt war. Er schloß daraus, daß ein Jagdrevier täglich neu erkämpft werden müsse (St. Quentin, 1934).

Als ich das gleiche Verfahren bei Untersuchungen an Libelluliden, vornehmlich an *Libellula depressa* L. und *Sympetrum vulgatum* (L.), anwandte, zeigte es sich, daß bei diesen Arten nach Entfernung des Revierinhabers das betreffende Revier einige Tage lang unbesetzt blieb. Erst nach dieser Zeit verschoben sich die Grenzen der Nachbarreviere so, daß nun der freie Raum von ihnen eingenommen wurde. Es scheint mir, daß dieser Befund ein wesentlich deutlicherer Hinweis auf Revierbesitz oder besser gesagt auf Territorialität ist, als die tägliche Neubesetzung der Territorien. Bei echter Territorialität muß ja gefordert werden, daß das Tier sein Territorium länger als jeweils nur einen Tag besetzt hält und verteidigt. Unter diesen Voraussetzungen kann man die Feststellungen St. Quentins nicht als Beweis für eine Territorialität werten, und es erhebt sich die Frage nach den Unterschieden im territorialen Verhalten zwischen den Libelluliden und Ae. cyanea.

Die ersten Hinweise zur Klärung dieser Frage erhielt ich, als ich — aus anderen Gründen — ein kleines Altwasser in den Traunauen bei Linz während der ganzen täglichen Flugzeit der Odonaten unter Kontrolle hielt. Die Auswertung der gemachten Aufzeichnungen ergab, daß während des ganzen Tages ein ♂ von *Ae. cyanea* an dem Gewässer flog. In Zeitabständen von durchschnittlich 35 Minuten tauchte jedoch ein anderes Exemplar für einige Minuten auf und es kam zu heftigen Kämpfen. Die gleiche Beobachtung konnte in einem anderen Biotop an *Aeschna grandis* (L.) gemacht werden. Als knapp nach einer solchen kurzen Kampfperiode die Platzlibelle gefangen und aus ihrem Territorium entfernt wurde, blieb das Gebiet, bis der Zeitpunkt für das Eintreffen des zweiten Tieres nach dem festgestellten Rhythmus gekommen war, unbesetzt. Ab da flog wieder dauernd ein Tier und die bereits geschilderten Vorgänge wiederholten sich bis zum abendlichen Ende der Flugzeit.

Eine eindeutige Klärung der hier herrschenden Verhältnisse konnte aber nur von Beobachtungen an markierten Tieren erwartet werden. Eine Markierung durch Anbringung von Farbflecken an gefangene Tiere erwies sich bereits bei früheren Versuchen als unmöglich, da diese Tiere nach ihrer Freilassung sofort ihr bisheriges Fluggebiet verließen und nicht wieder zurückkehrten. Es wurde daher die Methode von Moore (1952) in leicht abgewandelter Form angewendet und die fliegenden Tiere mittels einer Spritzpistole, wie sie als Kinderspielzeug in verschiedener Ausführung auf dem Markt ist, mit Farbe besprüht. Die Versuchstiere wurden dadurch nicht gestört, wenn auch in einigen Fällen Abwehrreaktionen in der Form einer starken Einkrümmung des Abdomens beobachtet wurden. Als Farbe wurden in Alkohol gelöste Holzbeizen verwendet. Als Nachteil wirkte sich aus, daß die Farbverteilung am markierten Tier vom Zufall abhängig war und daß daher Farben die am Tier von Natur aus vorkommen, nur beschränkt Verwendung finden konnten. Es ist also mit dieser Methode nicht möglich, eine größere Anzahl von Odonaten im gleichen Biotop zu markieren.

Um diesen Nachteil auszugleichen, wurde als Versuchsbiotop ein etwa 10 m² großer, sehr seichter Tümpel in einem engen bewaldeten Tal ausgewählt. Da das nächste Gewässer, an dem *Ae. cyanea* flog, mehr als 2 km entfernt außerhalb des Tales lag, war dort nur eine schwache Population zu erwarten. Tatsächlich wurden durch Markierung in diesem Gelände nur 5 ♂♂ von *Ae. cyanea* festgestellt.

Während der ganzen täglichen Flugzeit flog jeweils nur ein ♂ an dem Gewässer und hielt sich dort im Mittel 45 Minuten lang auf. Die Schwankungen der Aufenthaltszeit von 10—80 Minuten scheinen mit der Lufttemperatur im Zusammenhang zu stehen. An kühlen Tagen war die Aufenthaltszeit des Einzeltieres jedenfalls bedeutend kürzer als an heißen Tagen.

Eine bestimmte Reihenfolge der einzelnen Tiere konnte nicht festgestellt werden. Wenn ein Tier das Revier übernommen hatte, so wurde in der ersten Zeit seines Aufenthaltes jeder ankommende Artgenosse heftig angegriffen und verjagt. Diese Angriffe erfolgen ausnahmslos von unten her gegen den Thorax und werden mit der gleichen Bewegung ausgeführt, die auch beim Beutefang zu beobachten ist (Mayer, 1957). Während der ersten Aufenthaltszeit erfolgten die Angriffe der Platzlibelle so heftig, daß das fremde Tier stets die Flucht ergriff und ein Stück über die Grenze des Revieres hinaus verfolgt wurde. Dabei flog der Verfolger stets tiefer, d. h. in Angriffsposition. Je mehr die Zeit fortschritt, desto weniger energisch wurden die Angriffe der Platzlibelle, so daß die Neuankömmlinge zwar von ihr zuerst angegriffen wurden, jedoch versuchten ihrerseits anzugreifen. Da der Angriff grundsätzlich von unten her erfolgt, versuchte das angegriffene Tier seinerseits unter den Angreifer zu gelangen. Dieser pariert nun diese Versuche durch entsprechende Manöver und es entsteht der für den Luftkampf zweier Odonaten charakteristische Kurvenflug, der sich bis zu einem engen Abwärtstrudeln steigern kann.

Gegen Ende der Aufenthaltszeit der Platzlibelle werden die Angriffe auf neuankommende Artgenossen ganz schwach oder unterbleiben vollständig. Es kommt nun dazu, daß die Platzlibelle von einem Neuankömmling zuerst angegriffen und schließlich vertrieben wird. Nun übernimmt dieses Tier das Revier und die Kette der eben geschilderten Vorgänge beginnt von neuem. Wenn die Aufenthaltsdauer an kühlen Tagen gering ist, so fallen die Kämpfe weg, da in der kurzen Zeit kaum ein zweites Tier auftaucht. Es muß hinzugefügt werden, daß die tägliche Flugzeit an dem in Frage stehenden Biotop von 10—16 Uhr dauerte, vor- und nachher lag der Tümpel im Schatten der umgebenden Berghänge.

Wie eben geschildert, wurde im Normalfall die Platzlibelle durch den Angriff eines neu angekommenen Artgenossen veranlaßt, das von ihr bisher besetzte Gebiet zu verlassen. Um zu untersuchen, wie weit das Verlassen des Revieres unbedingt durch den Angriff eines Artgenossen ausgelöst werden muß, wurden alle neu ankommenden Tiere abgefangen, bevor es zu Kämpfen kam. Da alle diese Tiere auf bestimmten Wegen kamen und sich, bevor sie in das eigentliche Fluggebiet eindrangen, kurz an einem sehr kleinen, benachbarten Tümpel, an dem normalerweise keine Odonaten flogen, aufhielten, gelang das verhältnismäßig leicht. Die mittlere Aufenthaltszeit des Einzeltieres betrug an den Tagen, an denen diese Versuche angestellt wurden, 80 Minuten. Während dieser 80 Minuten blieben die Reaktionen der Platzlibellen, die nun nicht mehr in Kämpfe verwickelt wurden, durchaus normal. Nach dieser Zeit aber begannen die Tiere immer mehr über die bisher eingehaltenen Grenzen des Gebietes hinauszufliegen. Ich möchte diese Flüge als Intentionen ansehen, die eine Abflugstimmung anzeigen. Nach rund 100 Minuten verließen die Tiere ohne äußeren Anlaß das Gebiet. Es wäre daraus zu schließen, daß normalerweise der nach Ablauf einer bestimmten Zeit erfolgende Angriff eines Artgenossens als Auslöser zum Verlassen des innegehabten Territoriums fungiert.

Während seiner ganzen Aufenthaltsdauer im Gebiet flog das ♂ von *Ae. cyanea* ständig die Fläche des Tümpels ab. Der Flug wurde häufig durch rüttelndes Stehenbleiben unterbrochen, jedoch ließ sich das Tier nie auf einen Halm oder Ast nieder, wie dies bei Libelluliden die Regel ist. Sehr häufig wurden im Rüttelflug die Ufer abgesucht und hier besonders jene erdig-moosigen Stellen, die von den ♀♀ zur Eiablage bevorzugt werden. Dagegen trat der Beutefang verhältnismäßig zurück. Erschien nun ein ♀ im Biotop, so wurde es sofort angeflogen und das ♂ versuchte, die Paarung einzuleiten. Dieser Anflug erfolgt stets von oben her und steht damit im deutlichen Gegensatz zu der Bewegung, mit der der Angriff auf ein anderes ♂ eingeleitet wird. Es erfolgte dann die Tandem-Bildung und in dieser Stellung flog das Paar ab. Das Revier blieb dann frei, bis das nächste ♂ auftauchte und es in Besitz nahm.

Alle die beschriebenen Verhaltensweisen können auch an größeren Biotopen, wo die Fluggebiete mehrerer ♂♂ in der Uferzone aneinandergrenzen, beobachtet werden. Allerdings kommen dort zu den beschriebenen Ablösekämpfen noch weitere Kämpfe, die entstehen, wenn sich zwei benachbarte Tiere an den Reviergrenzen treffen und die dazu führen, daß das im isolierten Kleinbiotop gewonnene Bild stark verwischt wird.

Zusammenfassend kann gesagt werden, daß zwei von den eingangs genannten Kriterien für Revierbesitz oder Territorialität bei *Ae. cyanea* verwirklicht sind: Abgrenzung und Verteidigung. Moore (1952) und Kormondy (1959) erklären zwar die Angriffe auf Artgenossen des gleichen Geschlechtes als versuchte Paarungseinleitung. Ich konnte aber schon früher (Mayer, 1957) zeigen, daß Angriff und Paarungseinleitung zumindest bei den Aeschna-Arten mit zwei verschiedenen Bewegungen ausgeführt werden, wobei es unmöglich ist, daß ein Tier aus der von unten her ausgeführten Angriffsbewegung in eine Praetandemstellung gelangt. Das dritte Kriterium, der längere Aufenthalt des Revierinhabers, erscheint jedoch bei *Ae. cyanea* nicht gegeben.

Ich möchte daher das beschriebene Verhalten nicht als echte Territorialität, sondern als Vorstufe zu einer solchen ansehen. Es kann auch kaum von einem Jagdrevier gesprochen werden, da tatsächlich die Tiere während ihres Aufenthaltes an dem Gewässer nur wenig jagen. Meiner Ansicht nach hat das beschriebene Verhalten den Sinn, den einzelnen ♂ für eine gewisse Zeitspanne einen Uferstreifen zu sichern, in dem es die das Gewässer aufsuchenden ♀♀ erwartet, um mit ihnen zur Paarung zu schreiten. Diese Vorstufe leitet dann zu einer echten Territorialität bei Libelluliden über, wie ich sie in noch unveröffentlichten Untersuchungen feststellen konnte.

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THE CLASSIFICATION AND NOMENCLATURE OF CALOPTERYGINE DRAGONFLIES

(Odonata: Calopterygoidea)

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The distinctness of calopterygine dragonflies was first recognized by Leach (1815) who erected the Genus *Calopteryx* (i. e., *Calepteryx*, emended to *Calopteryx* by Burmeister, 1839) for "those Agrionida with coloured wings", but listed no species by name.

No further generic division of these forms was made until Selys (1840) created Euphaea for "*Calopteryx holosericea* Burmeister", 1839 (cited in error for *Euphaea variegata* Rambur, 1842) and Libellago for *Agrion* (i.e., *Calopteryx*) *lineata* Burmeister, 1839, *C. fenestrata* Burmeister, 1839 and *Agrion fulgipennis* Guerin, 1831, and Charpentier (1840) erected *Epallage* for *E. fatime*, a new species. However, Burmeister (1839) had divided the species which he treated into two groups—the second of which he characterized by very broad, densely-veined and (in the male) dark-colored wings and which corresponds, more or less, to the modern Calopteryginae (*Calopteryx*, *Neurobasis*, *Vestalis*, etc.). His first group characterized by small, sparsely-veined wings often marked with a colored spot or one-cell wide marginal band, was further subdivided into four subgroups. The first subgroup containing the species *lineata* and *fenestrata*, may be considered a concept corresponding to the Chlorocyphidae, but the others were less distinct in terms of later classification of the calopterygines.

Rambur (1842) placed the 35 nominal species which he listed, with descriptions, in four genera—*Calopteryx* Leach, *Euphaea* Selys, *Rhinocypha* and *Micromerus*. Unfortunately, he omitted Libellago Selys, and placed the three species originally included in that genus in his new genera, *Rhinocypha* and *Micromerus*. He furnished diagnostic tables to

the genera of each family of Odonata (except the "Aeschnides"). The table for the "Agrionides" (containing all Zygoptera) was divided into two primary divisions (Agrionoidea and Calopterygoidea in modern terms) upon the basis of the number of antenodal cross veins. The four genera of the "Premiere Division", "A" of the table (Calopterygoidea), were separated by the number of costal antenodals, the form of the clypeus and the form (size, presence or absence in the male) of the pterostigma, but this can scarcely be called a classification of the genera. However, it did make use of some of the characteristics now considered to be of family rank and represented a certain advance over the Burmeister classification into groups and subgroups.

The first real classification of the group was made by Selys in the "Synopsis des Calopterygines", prepared with the collaboration of Hagen, and published in 1853. Selys and Hagen recognized 100 species which were arranged into categories of eight or nine ranks.

This hierachy of classification, within the subfamily, included divisions, sous-divisions, sections, sous-sections, légions, genres, sous-genres, groupes and (frequently) sous-groupes. These were used throughout the *Synopsis* usually even when the group was "unique" within the next larger category, with the groups of the first four categories numbered or lettered, but the remainder both numbered and named. This scheme of categories, except for sous-sections was repeated in the Monographie des Calopterygines, 1854, (really only a more detailed treatment of the contents of the *Synopsis*) and in a list of species in the Triosiemes Additions, 1873, with all categories named. (It was also used, except for sections and sous-sections in the *Synopsis*, 1854, and Monographie des Gomphines, 1858; in the Synopsis des Cordulines, 1871, the only super-generic categories used were légions, and in the Synopsis des Aeschnines, 1885, none were used.) The "sous-genres", rather than genres, of Selys correspond in almost every respect, including use in binomial names, to our modern use of genera; his "genres" may be considered the equivalent of tribes or cohorts, and, in fact, many have become subfamilies. His légions are more or less identical with our families, and as he used generic names for them, they became established under the accepted rules of nomenclature as family-rank names. In a certain sense, then, there has been little modification of his scheme of classification except the addition of newly-discovered forms and the elevation of all ranks to those of higher value. He considered the Odonata as a suborder of the Orthoptera, our suborders, Anisoptera and Zygoptera, as tribes, with two (*Libellulidees* and *Aeschnidees*) and one family (*Agrionidees*) respectively.

In the preliminary chart of classification and in the body of the Synopsis seven "Légions"—*Calopteryx*, *Hetaerina*, *Euphaea*, *Dicterias*, *Libellago*, *Amphipteryx* and *Thore*—12 "genres" and 25 "sous-genres" were defined. However, in a section of "Additions et Corrections" at the end, and in the Monographie the number of "Légions" was reduced to five by combining *Hetaerina* with *Calopteryx* and *Dicterias* with *Euphaea*.

The classification of the seven (later five) legions was based upon the (1) origin of the "deux secteurs de l'arculus", (2) the number of subcostal antenodals, (3) the form of the clypeus and (4) the form of the pterostigma. Although he published four papers (one of them in two parts) of Additions to the Synopsis (1859—1879), Selys made no further changes in the major divisions.

The number of known species (and genera) continued to grow, but no further discussion of super-generic classification or relationships was published until Needham's study of the entire order in 1903. Selys reviewed this increase in the number of known species several times. In the Monographie he included a review of the number of

described genera and following the reference to the work of Rambur, noted, "C'etait un grand progress". However, the mere increase in the number of described species and genera is not necessarily progress; even then there were many synonyms among the specific names, and Rambur's treatment of genera has led to synonymy (even reversal) in names from genus to family!

In his Synonoymic Catalogue of Neuroptera Odonata (1890), Kirby listed 33 genera and 243 species of calopterygines (but called the group Agrioninae as noted below). These are arranged in a single linear list in identical sequence with those of a Selysian list of 1873 and additions of 1879, with the interpolations of a few forms described between 1879 and 1890. The difference in number of species (243 compared with 176 of Selys) is due mainly to the elevation of Selysian races and varieties. There have been no further lists or discussions of the classification of the calopterygines separately since that time.

In 1893 Calvert gave a geneological chart of the relationships of the subfamilies of the Odonata in which the Agrionines and the Anisoptera were shown as arising directly from the calopterygines.

In his geneologic study of dragonfly venation, Needham (1903) gave no chart or direct statement to this effect but his discussion indicates that he believed the richly veined calopterygines formed the stem from which the other groups developed. He recognized three subfamilies, calling the Selysian legion *Calopteryx* (including *Hetaerina*), the Vestalinae, the legion Thore, the Thorinae and all others Epallaginae, but noted "series" within the latter group corresponding exactly to the legions of Selys.

The apex of studies of Odonata indicating the richly veined calopterygine stem as the origin of other Odonata was reached with Munz's Venational Study of the Sub-order Zygoptera (1919). He gave reduction in venation as one of the principal criteria by which more highly developed and specialized forms were recognized. He divided the Calopterygines into the same three subfamilies as had Needham, plus one based upon a recently described form. A complete classification of genera into series by means of a key, with added discussions and explanations, retained the basic Selysian groupings.

At about the same time, Kennedy (1920) developed the opposite idea—that sparse venation was the more primitive type in the Odonata. His conclusions of origins and relations were based upon a study of the penes, rather than wing venation. He recognized seven, and a questionable eighth, subfamilies of calopterygines. These were the five legions of Selys, with the addition of the Philoganginae (not definitely placed by Selys when he described the genus *Anisonevra* (= *Philoganga*) for Hagen's species *montana* in 1859—"Sa place dans la serie est encore doutense") and the Megapodagrioninae and possibly the Platystictinae transferred from the Agrionidae. He noted that the development of the different groups had been "radial".

Tillyard (1938—1940) in his Reclassification of the order Odonata (completed by Fraser after Tillyard's death) gave a classification in almost complete agreement with that of Kennedy. They traced the origin of the groups of calopterygines from "near" the agrionid stem, almost all separately. Although they placed the Megapodagrionidae and some other genera within the Agrioniodea (called Coenagrioidea) they expressed so much doubt of their exact relationship that there appears to be no real differences from Kennedy's views. They recognized six families, with the Hetaerininae and two other groups (of post-1853 discovery) as subfamilies. In his Reclassification (1957), Fraser appears to have made no essential change in this arrangement, although he considered the calopterygines to have been derived from the Zygopterous stem through the "Lestine complex" (to which he consigned the Megapodagrionidae) and erected a family Pseudolestidae as "an annectent between the Coenagrioidae (= Agrionoidea)

and Agrioidae (= Calopterygoidea)" noting that "I am of the opinion that the genera it contains, lie nearer to the Agrioidea". Most of these genera were drawn from the Megapodagrionidae. Although Fraser considered that he was presenting "a new classification", the following table shows that except for the creation of a few subfamilies, almost the only difference from the classification by Selys in 1853, when he alternated between five and seven legions is in nomenclature.

Agrioidea (Fraser, 1957)	"Calopterygines" (Selys, 1853)
Amphipterygidae	
Philoganginae	(position doubtful, 1859)
Amphipteryginae	legion Amphipteryx
Chlorocyphidae	legion Libellago
Heliocharitidae	legion Dicterias
Polythoridae	legion Thore
Euthorinae	
Miocorinae	
Polythorinae	
Epallagidae	legion Euphaea
Agriidae	legion Calopteryx
Caliphaeinae	(included in legion Calopteryx, 1859)
Hetaerininae	(legion Hetaerina)
Agriidae	(legion Calopteryx)

Although we may say, with some semantic license, that the classification of the calopterygines by Selys is still valid, the nomenclature is certainly far removed from the serene and uncomplicated position of 1853—1890.

The major problem involves the name of the entire group—whether Calopterygoidea, Agrionoidea or Agrioidea. This was thoroughly treated in my paper, "Nomenclatural Confusion in the Odonata; the *Agrion-Calopteryx* Problems", in 1954, and little if any additional data can be presented. However, the problems have not been solved yet and it appears to many of us that the only sensible solution may be the one proposed by Calvert, that of dropping the name *Agrion* and its derivatives and using *Calopteryx* and *Coenagrion*. "What *Calopteryx* is is known, what *Coenagrion* is is known. *Agrion* is the uncertainty. What an unforgivable crime it would be from the priorist's point of view, to drop *Agrion* altogether!" Certain authors are now following this practice and all Odonatologists should consider this solution.

Another source of confusion in family names arises from the adherence of certain authors to the rejected principle of selecting the oldest extant genus as type. Thus Heliocharitidae has been employed in place of the proper name Dicteriastidae (based upon legion Dicterias), because of page priority of Heliocharis over Dicterias, and Epallagidae in place of Euphaeidae for the same reason.

Two other nomenclatural problems, involving invalid genera which were used by Selys as legion names and thus became the types of families, have been satisfactorily solved. These are Thore, preoccupied, replaced by *Polythore* (Calvert, 1917) and *Libellago* Selys, 1853, preoccupied by *Libellago* Selys, 1840, replaced by *Chlorocypha* (Fraser, 1928; Cowley, 1934).

LA FAUNE ODONATOLOGIQUE DE LA TÊTE DES EAUX DE LA DIABLE

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La Diable est une rivière torrentueuse sur une partie notable de son cours; elle coule nord-sud dans le centre du parc du Mont Tremblant et déverse ses eaux dans la rivière Rouge à quelque 20 kilomètres au sud de ce parc. Ce n'est qu'en ces dernières années qu'on a ouvert une route carossable jusqu'à son réseau supérieur. Auparavant, chasseurs et pêcheurs se rendaient sur les lacs de la tête des eaux pour y exercer leur sport favori, mais ils devaient s'astreindre à de longs portages. Les uns, tels les membres du club «La Madelon» possédaient un petit camp pour se retirer; d'autres devaient dormir à la belle étoile.

En 1957, avec un collègue, nous avons séjourné durant deux périodes de 20 jours au lac du Diable et au lac Bâtiment et, en 1958, durant deux périodes de même durée au lac d'Herbes, qui est situé à peu près à la même latitude, mais plus à l'ouest. Ces séjours avaient surtout pour but l'étude de la faune odonatologique. C'est dire combien nous avons tâché de profiter de nos séjours non seulement pour accroître nos collections, mais aussi pour accroître nos connaissances de la distribution des diverses espèces, du genre d'habitats recherché par chacune, des mœurs des diverses entités, etc.

Dans les hautes Laurentides, on se fait d'ordinaire une excellente idée de la faune odonatologique d'un territoire donné en collectionnant entre le 25 juin et le 10 juillet, puis entre les 10 et 25 août. Des conditions atmosphériques favorables sont quasi essentielles au succès de l'entreprise, car plus que tout autre insecte, les libellules ou Odonates répondent à la chaleur et à l'insolation par un regain d'activités, des va-et-vient nombreux, ce qui favorise leur capture. A l'inverse, les temps pluvieux ou couverts, les froids persistants empêchent presque complètement toute activité et les chances de récolte. Nous n'avons guère été favorisé par la température au cours des mois d'août.

Les régions explorées

La région explorée en 1957, celle du lac du Diable, compte sept lacs principaux dont deux de belles étendues. Tous communiquent avec le lac du Diable qui donne en fait origine à la rivière du même nom. Ces sept lacs furent visités, et de même les ruisseaux qui servent d'émissaires. Des digues de castors existaient en plusieurs endroits; les unes étaient toute récentes, mais d'autres dataient de plusieurs années.

Ces digues à la sortie des lacs et le long des ruisseaux favorisent le pullulement des Odonates qui s'établissent en nombre dans les étangs de barrages ou sur les rives inondées des lacs. La collection autour de ces étangs est souvent difficile, mais avec de l'ingéniosité et un non-refus de se tremper jusqu'à la ceinture on réussit de belles récoltes. Explorer sept lacs et sept ruisseaux en une quinzaine de jours ne permet guère plus d'une visite sérieuse à chaque endroit si l'on tient compte des distances à parcourir, des soins à donner aux collections, des périodes ensoleillées, etc.

La région du lac d'Herbes, visitée au cours de 1958, comprend un plus grand nombre de lacs et ruisseaux, soit une vingtaine. C'est pourquoi malgré notre désir, plusieurs coins n'ont pas été explorés. Les récoltes effectuées donnent cependant, croyons-nous, une excellente idée de la faune odonatologique présente dans le territoire couvert.

Le temps mis à notre disposition dans un congrès comme celui-ci ne permet pas d'entrer beaucoup dans les détails. Après avoir caractérisé les deux séries de lacs et cours d'eau, nous nous contenterons de montrer les différences qui existent dans la faune odonatologique.

Les lacs de la région du lac du Diable ont, pour la plupart, des rivages rocheux; la zone herbeuse est fort peu étendue; les ruisseaux roulent leurs eaux parmi les cailloux;

le gravier ou le sable est peu abondant. Au contraire, dans la région du lac d'Herbes, la plupart des lacs ont des rives sablonneuses, les lacs sont peu profonds et les plantes aquatiques fourmillent. Dans ces conditions les différences entre la faune odonatologique des deux régions pourraient être grandes, sinon du point de vue nombre d'espèces, du moins dans les populations de certaines espèces plus particulières à un habitat donné.

Arrêtons-nous d'abord à la diversité générale des espèces entre les deux régions:

Région du lac du Diable	**Région du lac d'Herbes**
53 espèces	54 espèces

formées de 45 espèces communes aux deux régions
où toutes les familles sont représentées

Espèces particulières au lac du Diable	Espèces particulières au lac d'Herbes
Boyeria grafiana Wmsn.	*Boyeria vinosa* (Say)
Dromogomphus spinosus Sélys	*Gomphus scudderi* Sélys
Cordulegaster diastatops Sélys	*Ophiogomphus colubrinus* Sélys
Somatochlora williamsoni Walk.	*Ophiogomphus aspersus* Morse
Somatochlora cingulata (Sélys)	*Ophiogomphus rupinsulensis* (Walsh)
Libellula lydia Drury	*Hagenius brevistylus* Sélys
Leucorrhinia frigida Hagen	*Somatochlora albicincta* (Burm.)
Neurocordulia yamaskanensis (Prov.)	*Sympetrum vicinum* (Hagen)
	Nehalennia gracilis (Morse)

Au total cela donnerait 62 espèces pour ce territoire central du parc du Mont Tremblant. Par comparaison, avec un territoire situé 25 milles plus au sud et à une altitude de 1100 pieds et non 1500 et 1600 pieds, le territoire s'avère plus pauvre, moins varié. En effet, dans la région du lac Monroe, c'est 83 espèces que nous avons capturées.

Remontant davantage au nord, nous pouvons présenter la comparaison avec un territoire situé en Abitibi que nous avons étudié il y a plus de 15 ans. Nous avions alors récolté quelque 40 espèces. Les environs du lac Mistassini parcourus en 1953 nous avaient fourni également une quarantaine d'espèces.

Principales différences entre les deux séries de lacs

Nous n'avons pas la prétention d'avoir effectué un inventaire complet dans chacune des régions; nos séjours ont été trop brefs par rapport au territoire à couvrir, les conditions atmosphériques désavantageuses nous ont fait perdre plusieurs jours complets. Dans ces conditions, il est sans doute prématuré de conclure définitivement à l'aide de ces données. L'absence d'une espèce peut être due à l'insuffisance des observations et non à son absence réelle. La présence et l'abondance de certaines espèces ne sauraient toutefois conduire à de fausses interprétations ou conslusions.

Parmi les espèces rencontrées qu'à l'une ou l'autre des séries d'habitats les plus significatives parce qu'elles tiennent aux conditions différentes d'habitats, semblent les suivantes:

Boyeria grafiana, présent seulement à l'émissaire du lac du Diable, à un endroit où l'eau coule sur des cailloux, et *Neurocordulia yamaskanensis*, observé en bordure du lac du Diable, dans une région où la roche en place et les rochers affleurents sont nombreux, requièrent apparemment ce genre d'habitats pour survivre.

Au lac d'Herbes, la présence de sable ou gravier, soit dans les émissaires, soit en bordure des lacs, favorise le pullulement des Gomphidés principalement: *Gomphus spicatus*, *G. exilis* et *G. borealis*. D'autre part, *G. scudderi* abonde dans les ruisseaux à lit sablonneux et les *Ophiogomphus* là où le gravier remplace le sable.

Le *Nehalennia gracilis* qui a des exigences d'habitats très poussées, rives de lacs acides ou tourbeux, n'a été rencontré qu'en un seul biotope; c'était d'ailleurs le seul qui convenait parmi ceux que nous avons visités.

Espèces à leur limite de distribution septentrionale

Ici comme ailleurs, il nous faut faire part de notre ignorance. Trop de territoires n'ont jamais été visités par les entomologistes pour être catégorique dans nos avancés. Ce sont plutôt des hypothèses de travail que nous formulons qui vaudront aussi longtemps que des faits nouveaux ne viendront apporter un démenti.

En nous basant sur nos connaissances actuelles de la distribution des espèces, nous croyons significatives les récoltes suivantes qui présentent une extension septentrionale de l'aire de distribution déjà connue: l'*Agrion amatum*, le *Nehalennia gracilis*, le *Boyeria grafiana*, l'*Hagenius brevistylus*, le *Lanthus albistylus*, le *Dromogomphus spinosus*, l'*Ophiogomphus rupinsulensis*, l'*Ophiogomphus mainensis*, le *Neurocordulia yamaskanensis*, le *Sympetrum costiferum*, le *Sympetrum semicinctum*, le *Sympetrum vicinum*, le *Leucorrhinia frigida*.

Conclusion

D'une part, la multiplicité des lacs et cours d'eau et la variété presque infinie d'habitats qu'offrent les Laurentides, d'autre part, l'influence bienfaisante qu'exercent les castors sur cette diversification et multiplication des habitats aquatiques favorisent non seulement le pullulement des Odonates mais aussi leur diversité. Le territoire couvert par cette étude, d'une superficie inférieure à 100 kilomètres carrés, a permis de déceler 62 espèces. Des études plus poussées permettraient peut-être de porter ce nombre à quelque 70 espèces. Il serait sans doute intéressant de mettre en parallèle des études faites sur d'autres territoires.

AGE-DETERMINATION OF ADULT DRAGONFLIES (Odonata)

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Observations recorded during the last sixty years or so have made it clear that the adult life of the dragonfly consists of two distinct phases. During the first, which is typically spent away from the breeding site, dragonflies feed but do not exhibit sexual activity. During the second, they make frequent visits to water for reproduction. In those species which have been examined, gonads of newly-emerged individuals are in an undeveloped state, and they mature during the first week or so of adult life (4, 40). It is therefore appropriate that the first phase should be called the maturation period, and the second the reproductive period. The behaviour of adults differs in each of these two phases.

As early as 1900 it was known that adults left the breeding site immediately after emergence (13), and that immature individuals were characteristically found away from water (23). For their first flight, adults of *Anax imperator* Leach orientate so as to fly away from water, and for the next few days they avoid water when it is encoun-

tered; the end of the maturation period is marked by adults making several indecisive flights over water and finally remaining there (11). The maturation period, which may be shortened by higher temperatures (4), is of similar duration in Zygoptera and Anisoptera, and in nature may last about one to three weeks (2, 8, 10, 11, 18, 40). For many individuals, this phase can occupy a large proportion of the total adult life: in *Pyrrhosoma nymphula* (Sulzer), after 15 days' maturation, some adults may enjoy a further expectation of life of only 6 days (8). Emphasis has been laid on the importance of the maturation period as a dispersal stage (26), and it appears that migrating individuals may sometimes (14, 15, 16), though certainly not always (16, 22) be immature. Finally, it is in the reproductive period that such well-known activities as aggressive behaviour, courtship and mating occur.

These few examples show how important it is in field studies to be able to determine accurately whether or not a dragonfly has entered the reproductive period, an event usually marked by mating in the male, and by mating or oviposition in the female. The main purpose of this communication is to present a brief review of those characters which may prove of value in distinguishing immature and mature dragonflies.

Copulation can leave distinctive marks on females and occasionally on males. The inferior anal appendage of the male (particularly in the Aeshnidae) may leave characteristic impressions on the compound eyes (31) or on the occipital triangle (40) of the female, whereas in those Zygoptera which oviposit in tandem, the female thorax may afterwards bear a residue of the sticky secretion by which the male appendages were attached to it (37, 39). It has also been observed that the legs of the female may leave recognisable scratches on the dorsal surface of the abdomen of a pruinose male (31). It would be valuable to know to what extent "copulation-marks" (31) are reliable diagnostic characters, and whether such marks may sometimes be left on immature individuals with which others have unsuccessfully attempted to mate. That copulation-marks can be found on the compound eyes of some males (11) should however sound a note of caution against immediate acceptance of this character in all cases.

Females which have already oviposited can sometimes be recognised by the differential soiling of the distal abdominal segments (1, 2) or of the wings (3); and in *Aeshna viridis* Eversm. wing-wear has been associated specifically with damage arising from the habit of ovipositing in *Stratiotes* (28). It is probable that considerable experience of individual species would be necessary before such methods could be used with confidence and precision. It has recently been noted, however, that like certain other insects which lay successive batches of eggs, parous dragonflies can be recognised by the condition of the ovaries, since these contain pigmented follicular relics and are supplied by tracheae which are loosely-coiled (12). So far, these follicular relics provide the only unequivocal means of determining whether or not a female has oviposited, and as such they could be used to assess the reliability of other, less definite characters, which might be easier to use in field studies, and which might not necessitate killing the specimen.

Students of the Odonata have available several other external characters of which the reliability as age-criteria has yet to be determined precisely. Foremost amongst these are the striking developmental colour changes which occur on the body (1, 2, 4, 18, 21, 29, 33) and on the wings (3, 5, 6, 33). In addition, as has been noted in other insects, the texture of the cuticle of the body and wings (*vide* 33) can undergo marked changes with age.

A further character which might be investigated with profit is provided by the presence on adult dragonflies of hydrachnid mites (7). These parasites leave the water with the emerging dragonfly, and return to it again when their host does so (27). It has been suggested that in dragonflies these parasites could provide a useful character

for recognising immature adults, since their first opportunity to leave the host comes at the beginning of its reproductive period (9). On some dragonflies, for example *P. nymphula*, hydrachnid mites undergo well-defined changes in shape and size during the host's maturation period (27), and it is possible that these also could be used to tell the age of the dragonfly.

Future work may reveal other characters by which the age of adult dragonflies can be estimated. Two possibilities seem particularly worthy of attention. The first is that newly-emerged adults may retain remnants of the larval gut for a specified period (*vide* 34); and the second is that freshly-mated females may have in the genital tract an object comparable with the mating-plug of *Anopheles* mosquitoes (17).

Research along these lines cannot fail to be rewarding. Whereas marking studies (2, 8, 10, 24, 32) have done much to establish the temporal relationship between the different phases of adult life, there can be no doubt that it would be valuable to be able to determine the physiological age of unmarked specimens. It is likely that our understanding of several puzzling features of adult life would be much increased by the possession of such knowledge. In particular may be mentioned problems associated with short-range dispersal (2, 26); migration (19); gregarious behaviour (30, 41); and crepuscular feeding activity, both over land and water, in species which are also diurnal (11, 20, 35, 36, 38).

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SYMPOSIUM XV

TAXONOMIE DER ENTOMOPHAGEN INSEKTEN

LA TAXONOMIE DES INSECTES ENTOMOPHAGES

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Introduction

La connaissance des insectes entomophages, par leur biologie et le rôle qu'ils peuvent jouer comme parasites ou prédateurs des insectes nuisibles, a pris une très grande importance. C'est sur cette connaissance que se base la lutte biologique, dans son sens le plus large, c'est à dire du maintien ou du rétablissement d'un équilibre naturel entre les espèces. De nombreuses études ont déjà été faites dans ce sens, mais il reste encore beaucoup à faire et nous sommes encore loin de pouvoir répondre à tous les problèmes compliqués qui se posent.

La taxonomie se base actuellement presqu'entièrement sur les caractères morphologiques des insectes; elle a été établie peu à peu par des entomologistes de musées ou par des collectionneurs qui avaient en vue de placer les espèces étudiées dans des classifications aussi précises et naturelles que possible. Toutes les monographies sont basées sur des caractères extérieurs de différentes parties du corps, forme, structure, coloration. Il était et il est encore difficile de faire autrement et il faut reconnaître que pour la détermination des genres et espèces et leur classement dans les collections, ces caractères sont précieux et le plus souvent suffisants.

Mais à côté des taxonomistes de musées, il y a les écologistes qui envisagent les problèmes sous un autre angle. Et tous les spécialistes qui reçoivent du matériel à déterminer, obtenu dans des élevages et envoyé par des écologistes, se trouvent bien souvent devant des problèmes difficile à résoudre; mais ils savent aussi combien ce matériel, obtenu souvent en grand nombre, est précieux pour résoudre ces problèmes.

Il y a principalement la question des limites de variabilité des espèces. Comment peut-on décider, sur des caractères morphologiques et des exemplaires de collections, si on a devant soi une seule espèce très variable ou de nombreuses espèces voisines les unes des autres? Que sait-on sur la variabilité ordinaire de chaque espèce, sur la variabilité suivant les saisons quand il y a plus d'une génération, ou sur la variabilité suivant les hôtes chez les espèces polyphages? Il y a d'autres causes possible de variations, la région, le climat, la densité des populations, etc. Pour bien connaître une espèce, il faut distinguer les simples variations de taille, de structure ou de coloration (auxquelles il est généralement inutile de donner des noms sub-spécifiques), des races géographiques et des races biologiques. Quand on se trouve devant des séries morphologiquement semblables mais différent par la biologie, on hésite s'il faut les considérer comme des races ou comme des espèces biologiques. On parle alors de «sibling species»;

c'est un problème que seul l'écologiste peut envisager, mais dont il faut tenir compte en taxonomie.

Il y a bien d'autres questions concernant la taxonomie que vous connaissez. Mayr, Linsley et Usinger (1953) ont pu dire aux Etats-Unis: «Il y a une tendance réelle chez les taxonomistes de considérer leur matériel de plus en plus comme biologistes et de moins en moins comme catalogueurs de musées.» En Europe cette tendance n'existe guère encore, car les rapports entre systématiciens et écologistes sont souvent trop peu fréquents. Celà tient à plusieurs causes dont la principale est que les taxonomistes de musées sont le plus souvent trop peu nombreux et surchargés de travail de déterminations et de classement, qu'ils manquent de place pour faire des élevages, et que l'étude des spécimens morts qu'ils reçoivent ou qu'ils ont récoltés suffit à remplir tout leur temps. Mais ils n'ignorent pas les problèmes qui seront envisagés ici et ils savent qu'il y a encore énormément à faire pour mieux connaître les espèces qu'ils ont dans les collections.

Comme spécialiste des Chalcidiens, dont j'ai établi un catalogue complet, j'ait fait un relevé du nombre des espèces connues en Europe et les régions méditerranéennes, en ne tenant compte que des espèces considérées jusqu'ici comme valables, à l'exclusion des nombreux synonymes et de quelques vieilles espèces mal décrites et restées inconnues depuis plus de 100 ans. A côté j'ai compté le nombre des espèces dont les hôtes sont connus, bien que le plus souvent la seule indication soit: «obtenu de tel hôte», sans autre renseignement biologique. Du tableau que j'ai ici, par familles, relevons seulement

Chalcidoidea

Nombre des genres et d'espèces paléartciques (Extrême Orient exclus). Relevé printemps 1960. Comptage exact des espèces valides, mais erreurs possibles dûes aux inconnues sur la validité de certaines espèces, aux nouvelles espèces peut-être pas notées et aux renseignements biologiques peut-être ignorés.

Familles	Genres	Total des espèces	Espèces dont hôtes connus	% spp. av. hôtes
Leucospididae	1	12	2	
Chalcididae	29	160	35	21,87
Podagrionidae	2	6	4	
Torymidae	23	245	156	63,67
Agaontidae	1	1	1	
Eurytomidae	10	242	123	50,82
Perilampidae	6	44	10	22,72
Eucharitidae	4	27	4	14,81
Eupelmidae	14	121	41	33,88
Encyrtidae	151	560	211	36,66
Thysanidae	5	9	6	
Aphelinidae	20	137	89	64,95
Pteromalidae (sens. lat.)	185	837	257	30,70
Cleonymidae	15	40	7	17,50
Elasmidae	1	17	10	58,83
Eulophidae (sens. lat.)	87	583	249	42,71
Trichogrammatidae	32	121	25	20,66
Mymaridae	37	436	32	6,86
Chalcidoidea, total	623	3598	1262	35,07

que sur le total auquel je suis arrivé, de 3598 espèces de Chalcidoidea paléarctitiques, seules 1262 espèces ont un ou plusieurs hôtes connus, soit 35%. Nous sommes donc dans l'ignorance complète de la biologie de près des 2/3 des espèces (v. tableau). Les familles les mieux connues en Europe au point de vue biologique sont les *Aphelinidae* (65% sur 137 espèces), les *Torymidae* (64% sur 245 espèces) et les *Eurytomidae* (51% sur 242 espèces); puis viennent les grandes familles, avec moins de la moitié des espèces avec hôtes connus: *Eulophidae* (43% sur 583 espèces), *Encyrtidae* (38% sur 560 espèces), *Eupelmidae* (34% sur 121 espèces) et *Pteromalidae* sens. lat. (30% sur 837 espèces). A la fin viennent les *Eucharitidae*, qui sont des parasites de fourmis, mais dont on ne connaît les vrais hôtes que de 4 espèces sur 27 en Europe, soit 15%, et les *Mymaridae*, parasites d'œufs d'insectes, dont seulement 7% ont leur biologie connue, c'est à dire 32 espèces sur 436 décrites.

Ma conclusion sera, au moins pour les Hyménoptères parasites, qu'il est actuellement plus important de trouver l'hôte ou les hôtes d'une espèce déjà connue, que de décrire une nouvelle espèce sur du matériel capturé au filet, surtout, sauf exceptions, si l'espèce est basée sur un ou deux exemplaires.

SULLA VARIABILITÀ DEI CARATTERI NEGLI HYMENOPTERA CHALCIDOIDEA

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La conoscenza della variabilità dei caratteri, fondamentale in ogni lavoro di sistematica zoologica, poco conosciuta nel gruppo in argomento, assume un particolare interesse per gli Imenotteri parassiti. Infatti essi comprendono forme che presentano grande variabilità e forme le quali, se confrontate secondo tradizionali metodi tassonomici, appaiono identiche tra loro sebbene distinte per caratteri biologici. Il seguente esempio dimostra che la tassonomia va riveduta secondo nuovi criteri che tengano nel dovuto conto la variabilità dei caratteri da un lato e dall'altro gli elementi che su di essa influiscono.

Secondo Schmieder (1933) la femmina di *Melittobia chalybi* Ashm. depone nello stesso ospite, a distanza di tempo, due diverse ovature; le larve che nascono dalla prima si nutrono della vittima ancora vivente, mentre quelle nate dalla seconda si sviluppano a spese dei resti lasciate dalle larve che le hanno precedute. Gli adulti che si evolvono dai due gruppi di uova presentano forme così diverse che un tassonomista che segue criteri tradizionali potrebbe considerare specie distinte se ne ignorasse la biologia.

Un esempio opposto dimostra come in specie così dette «gemelle», si possano trovare, con metodi di statistica biometrica e con la ricerca di nuovi caratteri, chiare differenze morfologiche. Mi riferisco al caso dei *Thysanus* del gruppo *subaeneus* da me studiato nel 1955.

Sulla variabilità dei caratteri nei *Chalcidoidea* riferirò quanto di più rilevante è stato posto in luce e quanto io stesso ho potuto osservare nel corso di questi ultimi anni.

Un capitolo importante riguarda le variazioni di colore. Ho potuto mettere in evidenza (1952), in uno studio effettuato per alcuni anni su di un Ecirtide, l'*Anagyrus pseudococci* Gir., che le femmine adulte, i cui stadi preimmaginali svernano, presentano

una colorazione bruna, quelle delle generazioni estive presentano colorazione in gran parte giallo arancio e quelle delle generazioni autunnali, parti del corpo brune e parti giallo arancio, cioè a colorazione intermedia tra le forme della generazione ibernante e quelle delle generazioni estive. Anche Moursi (1948), su due specie egiziane di *Anagyrus*, ha notato una variabilità stagionale dei caratteri colorimetrici che, pur non essendo ben specificata, si può ritenere analoga a quella da me osservata.

Ho notato tale graduazione stagionale del melanismo anche tra gli *Eurytomidae* ed *Eulophidae*. Gli adulti di *Eudecatoma biguttata* (Swed.) i cui stadi preimmaginali hanno svernato, si presentano pressocchè neri, mentre quelli delle generazioni estive sono quasi interamente giallo oro.

Il *Colpoclypeus florus* (Walk.) presenta nelle forme ibernanti l'addome completamente nero e parte delle zampe scure, nelle forme estive una macchia basale sull-addome biancastra e le zampe gialle.

Quale l'elemento determinante la maggiore o minore intensità del melanismo negli Imenotteri? Per citare dei dati sperimentali debbo riferirmi alle ricerche di Schlotte (1926) e di Lund (1934).

Sperimentando su *Bracon hebetor* Say, Schlotte ha ottenuto, variando la temperatura durante lo sviluppo pupale, individui giallo oro con temperature elevate, bruno scuro con temperature basse, parti di colore giallo e parti di colore bruno usando temperature intermedie.

Lund ha trovato, allevando differenti forme di *Trichogramma* che una di esse, allevata ad alte temperature, sviluppa femmine adulte gialle, mentre un'altra forma mantiene sempre femmine adulte brunastre.

Kerrich (1959), in una relazione sullo stato della sistematica degli Imenotteri parassiti ha posto l'accento sulla variabilità dei caratteri di colorazione indotta dal clima, tra i cui elementi ha certo preponderanza il fattore temperatura nell'indurre variazioni di colore.

In *Eudecatoma biguttata*, *E. variegata* (L.) ed *E. flavicollis* (Walk.) ho osservato che gli esemplari delle generazioni estive nel Nord Europa presentano limitate zone del corpo giallo, mentre tale colore è ampiamente diffuso negli esemplari allevati nel Sud d'Italia nel periodo corrispondente. Boucek (1959) riscontra in varie specie di *Cirrospilus*, colorazioni scure in esemplari di regioni nordiche e colorazioni chiare in esemplari del Sud Europa.

L'influenza della temperatura sulla melanogenesi è assai diffusa negli animali ed è ben nota; nel fenomeno generale va inserito anche l'ordine degli Imenotteri. Tuttavia i caratteri colorimetrici, considerati nel quadro della variabilità della specie, possono essere utilizzati nelle diagnosi tenendo conto della distribuzione dei colori sulle diverse parti del corpo.

Per quanto riguarda le dimensioni, non sono rari i casi in cui in una specie vi sono individui lunghi il doppio di altri. Ciò si verifica tra i *Leucospidae*, *Chalcididae*, *Pteromalidae*, *Eupelmidae*, *Thysanidae*, *Trichogrammidae*, *Eulophidae*, *Spalangidae*, *Eurytomidae*, *Torymidae*, *Ormyridae*.

Il rapporto lunghezza—larghezza del corpo nelle specie che non variano molto di dimensioni ha generalmente modesta variabilità: ma in un Tisanide, il *Thysanus ater* Walk., presenta differenze così notevoli in tale carattere che le forme estreme, ad un primo esame, appaiono come animali assolutamente diversi tra loro. Se però si analizzano tutti i singoli caratteri ad eccezione di quello citato, questi risultano perfettamente identici, compreso l'organo copulatorio maschile.

I caratteri del capo sono frequentemente ritenuti come poco variabili e presi come discriminanti. Gli studi sui Calcidoidei sicofili effettuati da Grandi (1921—1932)

dimostrano che tali caratteri possono essere notevolmente variabili. Anche in specie appartenenti a varie famiglie (*Encyrtidae*, *Perilampidae*, *Chalcididae*) il rapporto lunghezza—larghezza del vertice della fronte, il profilo di quest'ultima e la posizione degli ocelli possono variare alquanto.

Nel torace, il pronoto, nelle specie nelle quali è molto sviluppato, può presentare una notevole variabilità e, sovrapponendosi più o meno al mesoscuto, può apparentemente alterarne le proporzioni lunghezza—larghezza.

Un fatto analogo si verifica tra il mesoscuto e lo scutello negli *Encyrtidae*. In questa famiglia il mesoscuto può sovrapporsi appena, con il margine posteriore, allo scutello ed alle ascelle: il noto mesotoracico è diviso trasversalmente in due pezzi articolati da speciali processi endoscheletrici (Domenichini, 1954) che consentono al mesoscuto di sovrapporsi più o meno al margine anteriore dello scutello e delle ascelle. A seconda che tale sovrapposizione sia maggiore o minore le ascelle possono risultare a contatto tra loro o più o meno separate.

Il gastro presenta per lo più caratteri infraspecificamente poco variabili: la conformazione dei tergiti, la loro lunghezza e larghezza sono tali. Determinate condizioni fisiologiche dell'individuo, nonchè la morte e il disseccamento, possono però farla apparentemente variare.

Nelle femmine, la lunghezza della parte sporgente della terebra è talora assai variabile in specie che la possiedono di notevoli proporzioni: *Eupelmidae*, *Torymidae*, *Encyrtidae*, *Eulophidae*, *Leucospidae*.

L'armatura genitale dei maschi non presenta variabilità notevoli e potrebbe essere utilizzata maggiormente di quanto lo sia stato fino ad ora, come carattere interspecifico discriminante in numerose famiglie.

Delle formazioni endoscheletriche, nel capo, il tentorio (insieme con il ponte ipostomale) si presenta di forma costante come carattere infragenerico in molti *Encyrtidae* e potrebbe essere utilizzato nello stabilire rapporti filogenetici fra vari gruppi sistematici. Al contrario la lunghezza e la larghezza del postfragma e le dimensioni della parte dell'armatura genitale femminile introflessa nell'addome sono notevolmente variabili nell'ambito dei *Thysanidae*, *Aphelinidae* e *Trichogrammidae*.

Delle appendici le antenne dei due sessi sono da tempo considerate di importanza tassonomica fondamentale nei *Chalcidoidea*, tanto che, a volte, una sola piccola differenza nella loro struttura è bastata per far considerare una specie come nuova, che era tale solo per l'autore. Per quanto conosciamo le antenne offrono buoni caratteri ma si è poco indagato sull'argomento. In *Philotrypesis*, come rivelano gli studi di Grandi (1921) vi è forte variabilità infraspecifica; anche i dati biometrici di von Rosen su *Ahlbergiella aequa* (Walk.) mostrano una variabilità considerevole nelle antenne dei due sessi; in *Anagyrus pseudococci* è sensibile la variabilità nel rapporto lunghezza—larghezza di ciascun articolo antennale, ma non i rapporti tra le dimensioni di un articolo rispetto ad un altro. Invece in *Thysanus subaeneus* (Foerst.) sia nel maschio che nella femmina i rapporti tra la lunghezza della clava e la lunghezza dello scapo sono pressocchè costanti: lo scostamento quadratico medio è appena del 2,3% nella femmina e del 3,8% nel maschio (Domenichini, 1955).

Nelle zampe vi sono alcuni segmenti che sono di volta in volta di proporzioni variabili o costanti; le loro appendici e i processi tegumentali sono invece spesso molto variabili e molto comune è la asimmetria sia del loro numero che delle loro dimensioni.

Nelle ali si è dato grande importanza alle venature della ali anteriori, alla loro forma ed ai rapporti delle loro dimensioni. Come per le antenne si è non di rado ecceduto nel considerare rigorosamente costanti tali caratteri: lo rivelano gli studi biometrici su *Anagyrus pseudococci* (Domenichini, 1952), *Ahlbergiella aequa* Walk. (van Rosen, 1956), *Trigonoderus princeps* West. (Kerrich, 1957).

La chetotassi e la tricotassi sono state in generale usate limitatamente agli *Aphelinidae*, *Trichogrammidae*, *Thysanidae* ed in alcuni generi di *Eulophidae*. La distribuzione di peli e setole sia sul corpo che sulle ali merita uno studio più approfondito.

La scultura del corpo è pure di interesse tassonomico, anche se non di rado infraspecificamente variabile. Ma la distinzione dei generi sulla base di tali caratteri, come è stato fatto in certi *Pteromalidae*, può condurre ad errori.

Naturalmente non si può generalizzare; ogni carattere deve essere studiato specie per specie. Anche nella variabilità e nella stabilità dei caratteri vi sono convergenze e divergenze perfino nell'ambito di un medesimo genere.

I fattori che influiscono sulle variazioni morfologiche di questi artropodi possono essere numerosissimi; oltre ai fattori che influiscono sugli altri insetti, sui parassiti possono influire molti elementi inerenti l'ospite come: la specie ospite e il suo alimento, le sue dimensioni, il suo stadio, le sue condizioni fisiologiche, l'epoca in cui si svolge la generazione dell'ospite stesso. Oltre a ciò possono influire i fattori inerenti il tipo di parassitismo praticato e quelli inerenti la competizione parassitaria.

Noi possiamo dire che sia nel campo della variabilità dei caratteri che sulle cause che su di essa influiscono, quanto è stato fatto è ben poco rispetto a quanto rimane da fare. La sistematica di questa Superfamiglia, di cui conosciamo un numero di specie certamente modesto rispetto a quello che resta da scoprire e descrivere e nel quale le specie conosciute lo sono, in grande maggioranza, soltanto attraverso pochi individui, è, per la maggior parte dei gruppi, soltanto agli inizi.

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RECENT DEVELOPMENTS IN SUBFAMILY CLASSIFICATION OF THE ICHNEUMONIDAE

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The detailed study on the subject will be published elsewhere.

DIE BEDEUTUNG DER HABITATIO TYPICA FÜR DIE SYSTEMATIK ENTOMOPHAGER INSEKTEN

(12. Beitrag zur Kenntnis der paläarktischen *Aphidiinae*.)

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Die steigenden Anforderungen, die von Seiten der Praxis an eine systematische Bearbeitung wirtschaftlich wichtiger Insektengruppen gestellt werden, haben zu einer weitgehenden Umgestaltung und Verfeinerung der taxonomischen Methodik und zu einer stärkeren Berücksichtigung ökologisch-physiologischer Charakteristika geführt. Die Beschreibung einer Spezies lediglich nach ihrer äußeren Morphologie erwies sich vielfach als unzureichend. Die Definition der Art als Fortpflanzungsgemeinschaft mußte zwangsläufig zu einer Suche nach populationsspezifischen biologischen Merkmalen führen. Daraus folgt, daß der Typus als Individuum nicht allen Anforderungen, die an ein Richtmaß der Spezies gestellt werden, genügen kann.

Auf dem Gebiet der Entomophagensystematik bot sich die Wirtsbindung als biologisches Kriterium zur Abgrenzung systematischer Einheiten an. Ob und vor allem in welchem Umfang bei parasitischen Hymenopteren eine Spezialisierung auf bestimmte Wirtsgruppen stattgefunden hat, kann hier nicht im einzelnen erörtert werden. Das Phänomen der Wirtsspezifität ist zu mannigfaltig und von zu vielen Faktoren abhängig, als daß ein zusammenfassendes Urteil möglich wäre. Ich schränke deshalb die Gültigkeit der nachfolgenden Ausführungen auf die relativ kleine Gruppe der eigentlichen Blattlaus-Schlupfwespen (*Hymenoptera: Braconidae*, *Aphidiinae*) ein. Sie basieren auf der Auswertung zahlreicher Einzelzuchten, insbesondere aber auf der vergleichenden Untersuchung des Parasitenspektrums verwandtschaftlich zusammengehöriger Blattlaus-Gruppen.

Unter der „Habitatio typica" eines Parasiten verstehe ich die Wirtsangabe der typischen Zuchtserie. Der Ausdruck selbst geht zurück auf die Formulierung älterer Autoren „habitat inter ...", wurde von mir jedoch analog der Bezeichnung Terra typica, Locus typicus usw. umgebildet. Bei der Revision verschiedener Gattungen der Unterfamilie *Aphidiinae* wurden in erster Linie Tiere aus homogenen Zuchtserien mit bekannten ökologischen Daten herangezogen und an diesem Material die Variationsbreite der einzelnen taxonomischen Kriterien ermittelt. Dabei zeigte sich, daß eine nach typologischen Gesichtspunkten abgegrenzte Form in ökologischer Hinsicht stets auf eine kleine, verwandtschaftlich zusammengehörige Wirtsgruppe beschränkt war. Die hieran anknüpfende Überprüfung des Wirtsspektrums einer Parasitengattung, bzw. des Parasitenspektrums einer Blattlaus-Familie erlaubte die Annahme einer parallelen Evolution von Wirts- und Parasitenorganismen im Sinne der Fahrenholzschen Regel (Mackauer 1960b). Daraus ergibt sich, daß die Wirtsangabe einer homogenen Zuchtserie eine sichere systematische Zuordnung zu einer bestimmten Parasitenart gestattet.

Hierzu folgende Beispiele: Die Wirte der typologisch definierten Art *Lysiphlebus fabarum* (Marshall) s. l. gehören den 4 Subtriben *Aphidina*, *Rhopalosiphonina*, *Brachycaudina* und *Dactynotina* an. Von diesen sind *Aphidina* und *Rhopalosiphonina* verwandtschaftlich benachbart, während irgendwelche engere phylogenetischen Beziehungen zu den beiden übrigen Subtriben bis jetzt unbekannt sind. Der Verdacht lag nahe, daß unter dem Namen *Lysiphlebus fabarum* mehrere „sibling species" oder doch ökologisch differente Rassen zusammengefaßt sind. Der eingehende Vergleich der *Aphidini*-Parasiten mit den aus *Brachycaudina* gezüchteten Schlupfwespen erbrachte dann einen

zwar geringfügigen, aber konstanten morphologischen Unterschied und erlaubt so die Abgrenzung dieser letzteren als selbständige Art. Weiter war es möglich, die von Smith (1944) aufgestellte Art *Aphidius* (*Lysiphlebus*) *knowltoni*, in deren Wirtsverzeichnis sowohl *Aphis*-Arten als auch Vertreter der Gattung *Microsiphum* Chol. angeführt werden, in zwei sicher verschiedene Spezies aufzuspalten. Von Starý (1959) werden neben *Nasonovia*-Arten auch *Myzus ligustri* (Mosley) als Wirt der Schlupfwespe *Monoctonus crepidis* (Haliday) genannt. Der Wirtsbereich dieser Art ist jedoch auf die Gattung *Nasonovia* Mordv. und nahe verwandte Genera wie *Hyperomyzus* Börner beschränkt. Dagegen werden die Gattungen *Myzus* Pass. und *Ovatus* v. d. Goot von der *crepidis* sehr ähnlichen Art *Monoctonus cerasi* (Marshall) befallen.

Neben der Aufspaltung in mehrere Arten sind auch zahlreiche Fälle anzuführen, in denen bislang getrennt gehaltene Spezies mit gleicher Habitatio bei einem genauen Vergleich synonymisiert werden mußten. Die angegebenen Differentialkriterien erwiesen sich als unzureichend oder nur als populationsspezifisch. Dies gilt z. B. für die Arten *Praon longicorne* Marshall, *Aphidius gregarius* Marshall, *Aphidius granarius* Marshall, *Lysiphlebus chrysoaphidis* (Smith), *Paraphidius albiflagellaris* Stary, *Trioxys utilis* Muesebeck.

Die Bewertung der Habitatio typica als ein die Art kennzeichnendes Kriterium, hängt ab von dem Nachweis einer begrenzten Wirtsbindung. Diese Annahme kann für die Unterfamilie *Aphidiinae* als weitgehend gesichert gelten. Die Eroberung völlig neuer Wirte durch eine mutative Veränderung des Wirtsbereiches ist zwar möglich, stellt unter natürlichen Bedingungen jedoch eine äußerst seltene Ausnahme dar. Auch in diesen Fällen muß zunächst geprüft werden, ob der potentielle Wirtsbereich nicht an und für sich breiter ist als angenommen worden war und lediglich der Faktor „host acceptance" eine durch Umwelteinflüsse bedingte Änderung erfahren hat.

Diese bis jetzt lediglich auf die Art bezogenen Überlegungen gelten in gleicher Weise auch für inter- und infraspezifische Einheiten. Die Mehrzahl aller Parasitenarten ist nach heutiger Auffassung in ökologische oder physiologische Rassen aufgespalten, welche sich bei experimenteller Untersuchung und unter Freilandbedingungen verschieden verhalten. Das in diesem Zusammenhang besonders interessierende unterschiedliche Verhalten gegenüber nahe verwandten Wirtsläusen kann bis zu einem gewissen Grad als „Prägung" der Imago durch den Wirt oder andere Umwelteinflüsse erklärt werden. Darüber hinaus ist zu berücksichtigen, daß die meisten Parasitenpopulationen in genetischer Hinsicht Deme darstellen und als solche die Entstehung öko-phänotypischer Varianten begünstigen.

Salt (1941) verlangte die Zucht von Parasiten aus bekannten und standardisierten Wirten, um die versuchsweise ermittelten biologischen Daten vergleichsfähig zu machen. Die experimentellen Voraussetzungen hierfür dürften, insbesondere bei der Bearbeitung einer Gattung oder höherer Einheiten, noch fehlen. Ein wesentlicher Fortschritt wäre jedoch schon dadurch zu erreichen, daß bei sämtlichen parasitischen Insekten, in erster Linie aber bei den Endoparasiten, die Habitatio typica der ursprünglichen Serie als „biologische Konstante" angegeben würde. Es ist durchaus möglich, derartige Angaben nach den Regeln der Offenen Nomenklatur als nähere Erläuterung an den Artnamen anzuhängen. Als Vorbild könnten vielleicht die Wirtsbereichsangaben dienen, wie sie in der Mikrobiologie zur Unterscheidung der einzelnen Zuchtstämme benutzt werden: Die typische Wirtsart würde durch ein Pluszeichen, die nicht angenommene Differentialart durch ein Minuszeichen gekennzeichnet werden. Es wäre vermieden, daß eine lediglich durch ihre „host preference" aber nicht morphologisch abweichende bionomische Rasse nomenklatorisch valent benannt würde. Es folgt weiter, daß zweckmäßigerweise nur noch die Angehörigen der typischen Zuchtserie und nicht mehr alle bei der Abfassung einer Neubeschreibung untersuchten

Exemplare als Paratypoide bezeichnet werden. Der zahlenmäßige Verlust begehrter Tauschobjekte wäre durch die erhöhte Aussagefähigkeit des paratypischen Materiales bei weitem ausgeglichen.

Abschließend seien einige wesentliche Vorteile genannt, die sich aus der Berücksichtigung der Wirtsbindung entomophager Insekten ergeben: Der Systematiker erhält ein zusätzliches Hilfsmittel zur Bestimmung schwieriger Arten, zur Klärung einer Species dubia durch Neuzüchtung oder zur Überprüfung systematischer Einheiten auf ihre Homogenität. Gleichzeitig kann er wertvolle Vorarbeiten für eine breitere Verwendung parasitischer Insekten im Rahmen der biologischen Schädlingsbekämpfung leisten.

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DIE NEUE SYSTEMATIK DER FORMICA RUFA-GRUPPE

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Die Systematik der Gruppe der *Formica rufa* ist schon lange rätselhaft. Die älteren Autoren (Schenck, Förster) waren überzeugt, daß mehrere Arten vorlagen. Mayr, der alte Großmeister der Ameisensystematik, betrachtete alle diese Formen als zu einer Art, *F. rufa*, gehörig. Nur die Art, die er *F. congerens* und wir nun *F. nigricans* Em. nennen, trennte er ab[1].

Im Jahre 1874 publizierte Forel seine „Fourmis de la Suisse". Seine merkwürdige und abweichende Auffassung der Artensystematik der europäischen Ameisen war unglücklicherweise bis vor kurzem maßgebend. Er unterschied Arten, die er wieder in Rassen unterteilte. Zwischen den Rassen glaubte er Übergänge erkennen zu können, zwischen den Arten jedoch nicht. Die Übergänge betrachtete er, wie aus seinen Ausführungen hervorgeht, als Hybriden. Zur Unterscheidung seiner Rassen gebrauchte er hauptsächlich Farbunterschiede und keine morphologischen Merkmale. Als Rassen betrachtete er *rufa* sensu strictu, *pratensis* und *truncicola*, als Übergänge nennt er *rufa-pratensis* und *truncicola-pratensis*. In seiner Bearbeitung der Ameisen der Schweiz (1915) behält Forel sein ursprüngliches System bei, nur erscheinen auch noch die alten Namen *piniphila* Schenck und *polyctena* Förster neben den alten Namen für die Übergänge. Es ist schon lange bekannt, daß es unmöglich ist, eine Ameise der *rufa*-Gruppe mit

[1] *F. truncorum* lassen wir im weiteren außer acht, weil sie nur eine untergeordnete Rolle gespielt hat.

Hilfe der Forelschen Tabellen zu bestimmen. Auch Forel konnte es nicht. Ich habe durch die Freundlichkeit des Herrn Dr. Ferrière einen Teil des Materials der *F. rufa-pratensis* Forels studieren können. Es stellte sich heraus, daß fast alle Arten der *F. rufa*-Gruppe darin vertreten waren. Unglücklicherweise haben verschiedene seiner Nachfolger versucht, die Tiere nach Forel zu bestimmen (Wasmann, Donisthorpe). Das Resultat war eine noch größere Verwirrung. Emery und Stitz konnten auch mit dem System Forels nichts anfangen. In Zweifel über die Richtigkeit des Forelschen Systems und in Ermangelung von Verbindungen mit dem Ausland hat Gößwald während des letzten Krieges ein eigenes Arbeitssystem aufgestellt (1942). Es war ihm deshalb nicht bekannt, daß schon verschiedene Arten der *F. rufa*-Gruppe genügend umgrenzt waren. Bondroit (1918) war der erste der modernen Autoren, der einige Arten richtig umgrenzte. Die boreo-alpinen Formen, die stark behaart waren, nannte er *F. rufa*. Die wichtigsten Arten aus der Norddeutschen Tiefebene nannte er *F. piniphila* Schenck und *F. polyctena* Först. Auch *F. pratensis* (nun *F. nigricans*) hat er richtig erkannt. Die Ameisenkenner seiner Zeit erklärten ihn fast für geisteskrank, mit dem Erfolg, daß Bondroit seine Arbeiten nicht mehr fortsetzte.

Zwei Holländer, Stärcke und Betrem, entdeckten (1928) fast gleichzeitig, daß das System Bondroits viel besser für die Bestimmung der Arten der *Formica rufa*-Gruppe brauchbar war, als dasjenige Forels. In ihren Publikationen bauten sie das System nun weiter aus (Stärcke 1944, Betrem 1953). Der letztere machte einen neuen Bestimmungsschlüssel für die Arten der *F. rufa*-Gruppe.

Yarrow aus dem „British Museum“ untersuchte die Richtigkeit der verschiedenen Namen und entdeckte, daß noch eine bisher nicht beschriebene boreo-alpine Art vorhanden war, die er *F. aquilonia* nannte. Durch die Untersuchungen von Yarrow wurde nun die Nomenklatur auf feste Grundlage gestellt. Durch einen Beschluß der Internationalen Nomenklatur-Kommission wurde fesgestellt, was *F. rufa* eigentlich ist. Es ist die *F. piniphila* Schenck, wie sie genannt ist von Bondroit, Stärcke und Betrem. Die *F. rufa* der drei genannten Autoren muß nun den Namen *F. lugubris* Zett. tragen. Yarrow kannte die *F. polyctena* Först. aber nicht. Betrem hatte jedoch seit 1928 betont, das diese eine gute Art sei. Durch seine Untersuchungen und später auch durch die von Lange ist die Richtigkeit dieser Auffassungen nun sicher gestellt. Wir kennen nun die Arten:

F. lugubris Zett.
F. aquilonia Yarrow
F. rufa L. 1761
F. polyctena Först.
F. nigricans Em.

Die Unterscheidungsmerkmale dieser Arten liegen wesentlich in der Behaarung. Es ist jedoch nicht immer leicht, die Zugehörigkeit festzustellen, weil die Intensität der Behaarung variiert. Es können Fälle vorkommen, in denen man über die Artzugehörigkeit im Ungewissen ist. Dies besonders dann, wenn man nur einzelne Individuen vor sich hat. Man muß darum Nestserien (mindestens 5—10 Arbeiterinnen) untersuchen. Besonders die alpinen Arten sind bisweilen schwierig zu unterscheiden. Man muß dann eine Tabelle vieler Merkmale der Individuen eines Musters machen. Dies ist jedoch eine zeitraubende Arbeit. Fast immer kann man dann sagen, welcher Art solch eine Probe angehört. Betrem (1958—1960) hat die Synonymie der Arbeitsbenennungen Gößwalds festgestellt, so daß man nun weiß, welche Arten damit gemeint sind. Die *minor*-Form ist meistens *Formica polyctena*. In derselben Arbeit Betrems ist die Geschichte der *F. rufa*-Gruppe ausführlich angeführt. Auch eine neue Bestimmungstabelle ist gegeben worden.

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TRICHOGRAMMA: A COMPLEX OF SIBLING SPECIES

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The genus *Trichogramma* is represented by at least six morphologically distinct species, *T. evanescens* Westw., *T. embryophagum* (Hartig), *T. semblidis* (Aur.), *T. minutum* Riley, *T. japonicum* (Ash.), and *T. retorridum* (Gir.). This conclusion is based on information reported by Muesebeck et al. 1951; Flanders 1935; Girault 1911; and Ferrière 1947. Many additional species, identifiable only biologically, may exist.

The larvae of *Trichogramma* in general are not highly specialized nutritionally. As pointed out by Brues (1908), some species exhibit almost as great a range in hosts as do the genera and even larger groups of other parasitic Hymenoptera. The *Trichogramma* adult, by its selection of host plant and subsequently of the host insect, has a greater limiting effect on the kinds of host insects successfully attacked than do the nutritional needs of its larvae.

Biological characters of systematic value include patterns of development, environmentally influenced developmental rates, adult coloration and longevity, the inability to interbreed, and the habitat (host plant) preferences of the gravid female. The taxonomic validity of *Trichogramma* species requires that description be based on the progeny of field-collected specimens, such progeny having completed development in a common host and at a constant temperature of 30° C.

Under field conditions, sibling species may be characterized by differences in their capacities to maintain their host populations at noneconomic densities. A field character of major importance is the type of habitat and the kind of host plant most frequented (preferred) by the gravid female (Flanders 1937). The exact identification of a *Trichogramma* species requires the use of most, if not all, the characters noted above.

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THE SIGNIFICANCE OF SOME SPECIFIC DIFFERENCES, WITH PARTICULAR REFERENCE TO EURYTOMA TIBIALIS Boh. AND E. SERRATULAE (Fab.)

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The major task of the taxonomist working with the Chalcidoidea is to provide descriptions and diagnostic characters for the species of his group. In fact we are following the same, though rather more refined methods as did Linnaeus himself. Thus, it is important to question the nature of the entities which we call species.

The essentially monotypic species of Linnaeus was characterized by a rather vague attribute of breeding true. Following from this, the only definite criterion now recognized for deciding the specific distinction of two forms is the presence of reproductive isolation in the field or the lack of what Mayr has termed crossability between them. Most of the problems resulting from the species concept in modern times have arisen from its extension in order to accommodate variation in space (geographical variation) and variation in time (phyletic evolution). I am not here concerned with either of these problems. I wish only to talk about sympatric forms: that is those forms whose breeding ranges either coincide, overlap, or at least meet over an extensive area. In theory there should be no problem in deciding the specific status of such forms. However, in practice few taxonomists are able to observe living animals and fewer to obtain field breeding data. Thus, we usually have to rely on morphological characters as indicators of specific distinction. Nevertheless such morphological differences are not necessary a priori requisites for the coexistence of distinct species, though usually they do exist.

Within the Chalcidoidea there are many groups of sibling species: that is species which differ only very slightly in normal museum material, but which represent quite distinct non-interbreeding entities. Biological studies on closely allied sympatric species (which may range from extreme siblings to forms with easily recognizable characteristics) should throw light on the true nature of specific differences and when used with caution on the process of speciation itself.

As an example I should like to consider two species of *Eurytoma* both of which are internal parasites of gall-forming Trypetid flies — *E. tibialis* Boh. (= *E. curta* Auctt. nec Walk.) and *E. serratulae* (Fab.) (= *E. tristis* Mayr). Adults are easily distinguished in the female sex by the distinctive shape of the gaster which itself is correlated with the considerably longer ovipositor of *E. tibialis* compared with that of *E. serratulae*. *E. tibialis* attacks a wide variety of Trypetid species, such as *Urophora stylata* (Fab.), all of which form hard woody galls in the flower heads of various Compositae. *E. serratulae* has been reared only from *Urophora cardui* (L.), a gall-former in the stems of *Cirsium arvense* (L.) Scop. It seems likely that oviposition in the developing stem-galls of *U. cardui* would not require as long an ovipositor as oviposition in the flower-galls attacked by *E. tibialis*. Thus the major morphological difference between the species may be correlated with different host preferences. However, this ecological separation would not prevent the two forms from interbreeding and thus the primary isolating mechanism operating between them must be looked for elsewhere—possibly in courtship behaviour. In this example the two species are clearly separated by distinct differences, but these differences can reasonably be correlated with host preferences. Thus it would be conceivable for the two forms to have identical gasters but still remain good species, in which case they would be extreme siblings and almost impossible to separate morphologically. Only detailed biological work can elucidate the true isolating mechanisms operating between species.

AN ASSESSMENT OF THE SIGNIFICANCE OF COLOUR IN THE SYSTEMATICS OF THE HYMENOPTERA PARASITICA

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It has long been recognised that most of the earlier systematists relied too much on colour characters, especially for the distinction of species, and the tendency during the last four decades has been to decry the use of colour characters in systematic work. Modern revisions, when undertaken, reveal a previous state of confusion greater than had generally been suspected. But such revisions are not yet available for many sections of the Hymenoptera, at least not on a continental scale; so that European workers are most frequently driven back to using the older monographs such as those of Schmiedeknecht, Fahringer and Kieffer. The work of C. G. Thomson was, of course, technically superior to any of these, but unfortunately it was much less inclusive.

In the monograph of Schmiedeknecht, the keys to genera are based almost entirely on structural characters, the keys to species mostly on colour: yet, in using these keys for determining species, one does reach the correct result surprisingly frequently. This can only mean that colour does have some value in the systematics of the group, greater than it has latterly been the fashion to assume, and it seems that the time has come to reassess the position.

If one considers the matter, one finds that colour has some significance at all systematic levels, but that most often there are important exceptions. (Here, examples were given of coloration characteristic of different systematic levels from superfamilies down to groups of species, sometimes in one sex only or in a geographical region, and of exceptions to these examples.)

It is on the question of the use of colour characters for distinguishing species that the greatest amount of heart-searching arises; yet we still use colour characters to a greater extent than many are willing to admit. Thus, females of the genus Ichneumon are divided into groups of species on the number of white spots near the apex of the gaster, and individual species are still distinguished largely by differences in the paler colour on the gaster and legs. These insects are often found in large numbers in hibernation, and do seem to separate into good species; and the practised specialist will appreciate a uniformity of structure within the species, even though this may not have been adequately defined.

It is necessary to discover by intensive study, and not just by superficial observation, what colour characters can be relied on for species determination and what cannot. Thus, the presence or absence of a conspicuous pale mark on the scutellum are alternative conditions in one species, but provide a constant difference between two closely allied species in a related genus. In another genus there are two species that superficially are very similar, yet have good structural characters to separate them. Both vary greatly in the degree of development of the red and yellow colouring, and in the extent to which these are overspread with infuscation. Yet there is an almost constant colour character in that one has the hind trochanter always dark-marked above, the other hardly ever so.

Overspreading colours may be very extensively developed in some specimens of a species, but show all gradations down to a mere trace. In these cases the positive character is the presence or absence of the colour and not its extent.

Some major difficulties may be recognised under different headings.

Geographical variation. It is generally recognised that insects living in warmer and drier climates tend to be lighter in colour than those living in cooler and damper places. This has both inter- and intra-specific significance. It has been found in some groups that colour characters used in dichotomous keys based on material from continental, especially southern, Europe do not hold for British material.

Melanic varieties. It has been found that in many species of Ichneumonidae, in which the gaster is broadly red, that there are varieties in which the red is wholly or mainly replaced by black, with very little intergradation. There is not yet enough information to say whether such distinct melanic varieties are commoner in damper climates.

Seasonal variation may be mentioned in passing.

Metallic colours: conditions of viewing. Some years ago I was studying three species of an Encyrtid genus, including one new. Timberlake had described the top of the head in one species as black, with a slight greenish lustre, and the other as much more distinctly greenish. I found the colour to be decidedly greenish in all species in ordinary illumination supplemented by good London daylight, but that the green appeared weaker in strong artificial light.

Perhaps we should set standard conditions for viewing and describing colours; but if we should rely on artificial light and exclude daylight as far as possible, what of the colours that do appear in daylight? Personally, I like to use good daylight, supplemented as little as possible by artificial light, for describing colours of medium sized Ichneumonids.

The species of the Eulophid genus Syntomosphyrum parasitic on tse-tse flies are instructive in two respects.

Waterston named the first species glossinae, and described it in detail running to four pages of text. The following year he received another form, but failed to distinguish this by any other character than that the female antennal club was white instead of fuscous. Yet recent investigation shows, on grounds of biology and distribution, that the two are to be considered as good species. From the difference in colour of the female antennal club, that would be expected; but no structural character has been found to hold good other than a slight difference in proportion of some female antennal segments.

It was thought there might be a difference in the general body colour, but this also breaks down. Moreover, the holotype appeared to me green when viewed at mid-day one day, but the green had changed to blue by 3.20 p. m. when daylight had largely faded.

In the systematics of Hymenoptera parasitica the study of colour should be subordinate to that of structure, and is much more liable to exceptions: but it has a rôle to play, which should not be neglected, and which, when used with experience and discrimination, can point the way to positively valuable results.

SYMPOSIUM XVI

ANGEWANDTE ACAROLOGIE

ZUSAMMENFASSENDER ÜBERBLICK

G. DOSSE

A third symposium on Acarology (Wageningen 1956, East Malling 1959) took place in Vienna at the occasion of the Int. Congress of Entomology 1960. At the outset Prof. Dosse mentioned that it is very much appreciated to meet with overseas colleagues and that the personal contact facilitates the co-work of all acarologues whose main problems are the same.

Dr. G. O. Evans first reported on phytophagous mites and pointed out the species conception. He stated that morphological studies alone cannot give a satisfactory answer to the problem, supplementary biological studies would always be necessary. This was confirmed by Dr. H. H. J. Nesbitt (Canada) and Dosse. Dr. Nesbitt agreed that the morphological character of one species may change if the species is reared for longer periods on different host plants. Dosse found individuals of *Typhlodromus tiliae* Oud. occuring on one apple leaf which could not be crossed. Dr. J. G. Rodriguez (USA) gave an account of his research on synthetic mediums to rear mites. If his attempts are successful, the influence of the host plant can be eliminated, resulting in pure and well-described clones (compare *Drosophila*). Dr. R. Gasser (Switzerland) asked if the rearing of the types can be done by research workers in the different museums on natural history. Evans suggested that this better should be done by biologists who are working with the different species. On the last symposium on acarology at East Malling it was proposed that this work should be carried out by Dr. N. W. Hussey (England) for the *Tetranychus urticae*-complex. Evans wants to avoid the unnecessary creation of new species by introducing the word "forma" plus the host plant, e. g. *Bryobia praetiosa* Koch forma from apple. Dr. R. L. Beard (USA) did not agree with these ideas and stated that 100 years ago the same problem existed in general entomology. This problem now is worked out successfully without "forma's". Van Eyndhoven (Holland) pleaded for the old nomenclature and that one should try to find the species in question at the place of its original provenance. Evans and Nesbitt, however, did not agree to this idea. Evans then discussed the nomenclature. He suggested to ask the International Committee for Nomenclature to standardise certain names of mites of economic importance. So the undesired change of the names of these mites which easily leads to confusion, can be avoided. As an example he mentioned the fruit tree red spider. In USA it was called *Paratetranychus pilosus*, later on *Panonychus ulmi* and in the near future this name will be changed again, while in Europe it is known by Metateranychus ulmi. Further examples concerning the names of mite-species of economic importance can easily be found.

Dosse reported on his research on the feeding of predatory mites with pollen. Although the reproduction of *Typhlodromus tiliae* was hampered, this predatory mite can be kept alive for a long time when fed with pollen. As shown by laboratory investigations it is possible to feed *T. tiliae*, just appearing from its winter quarter, with fresh pollen and to produce some eggs. By this fact a start for a new population is given and the mites may survive during the blossom period of fruit trees, when no phytophagous mites are present. Nesbitt stated that in Canada *Typhlodromus* mites feed the winter eggs of *M. ulmi* while Dosse never observed this in Germany.

Dr. Gasser informed on the resistance of mites to insecticides and acaricides. He stated that if a population of mites shows resistance to a certain chemical it also is resistant to chemicals that belong to the same group, e. g. the organic phosphorous insecticides. This is confirmed by research work carried out in Holland by Dres. W. Helle and L. Bravenboer. On the genetical base of the resistance problem little is known up to now. Tests carried out some years ago by Dr. F. Smith (USA) showed that resistance was caused by one factor. Helle on the other hand found it to be more complicate and accused the responsability to multiples factors. If a population of mites is resistant to a certain group of chemicals, this resistance does not disappear. Smith got the same resistance rate even after 500 generations.

An interesting item was given by Dosse, who succeeded in inducing resistance of *Typhlodromus tiliae* to DDT by regular spraying of this insecticide on apple trees infested with M. ulmi and this predatory mite. By request of the chairman Bravenboer gave some details of his research with a new predatory mite, described by Dosse as *Phytoseiulus riegeli*. Because of its very short life-cycle (at 25—30°C only 3—4 days) this natural enemy may become of great importance in the biological control of mites, especially in glasshouses and perhaps also in tropical and subtropical areas. *T. urticae* gave in preliminary experiments a population density of over 100 individuals per leaf within one month. Especially on crops where beside mites only few pests or diseases have to be controlled, this predatory mite may be very useful in a combined biological and chemical control of mites.

The papers of Evans, Dosse and Gasser will be published elsewhere in a due time.

PRELIMINARY STUDIES ON BIOLOGICAL CONTROL OF HOUSEFLY EGGS USING MACROCHELID MITES

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Some of the Acarina have been noted to be associated with the housefly *Musca domestica* as predators. Macrochelid mites appeared in collections of pasture turf plugs in Kentucky, in rotting humus or manure. Collections made at housefly breeding sites within a 90-mile radius of Lexington have regularly yielded two species of *Macrocheles* living side by side, *M. muscaedomesticae* and *M. plumiventris*. They occur in the outer two zones of the manure pile, the biggest populations thus far having occurred in the spring when manure temperature was 28°C. Efforts at mass rearing of *M. muscaedomesticae* have been directed at finding a suitable substrate and optimum temperature,

moisture and p_H. Cattle manure, air dried and ground, combined with wood shavings has been the most satisfactory substrate base, which should be damp and at p_H 7. Both mite species thrive on fresh or frozen housefly eggs and will also feed on first instar maggots as well as to some extent on adults, which transport the mites. Natural foods such as fish eggs, egg yolk and skim milk have not proved to be better food than fly eggs, even with brewers yeast added. Livestock feed additives showing promise when added to the substrate include aureomycin, skim milk, fish meal and Pfizer vitamin supplement. Biological control experiments of an exploratory nature have been performed in the laboratory, and in the field with small cages sunken into manure piles. A search for a selective acaracide to eliminate naturally occurring mites in the cages revealed that Tedion is relatively safe to hatching fly larvae while moderately toxic to *M. muscaedomesticae*. In the laboratory, 91.5% control of maggots was obtained when the ratio of introduced mites to eggs was 1:5. Mites consumed about 5 eggs per mite in a 24-hr. period and the amount was doubled when the mites were previously starved for 48 hours. Housefly egg hatch in the laboratory averaged 24% with no mites present as compared to only 1% in the presence of mites at the ratio of 1 mite to 6 fly eggs.

SYMPOSIUM XVII

GRUNDFRAGEN DER SYSTEMATIK UND NOMENKLATUR

EINFÜHRUNGSWORTE

E. MARTINI †

Als bald nach 1945 Mitteleuropa von Antimilitarismus fast überfloß, hat ein englischer General gesagt, Kriege werde es so lange geben, solange es vorkomme, daß ein Staat kein Verständnis dafür aufbringe, was die unabdinglichen Lebensnotwendigkeiten eines anderen Staates seien. Das ist aber kein Privileg der Staaten. Überall, wo das gegenseitige Verständnis fehlt, ist Gefahr des Konfliktes. Es sei an die Revolte von Karl Escherich 1941/42 erinnert. Ziel: Eine stabile Nomenklatur für die praktisch zoologischen Fächer ohne Rücksicht auf Regeln und Kommission. Die theoretischen Fächer würden sich schon anschließen.

Dieser Vorgang hat heute glücklicherweise nur noch historisches Interesse. Inzwischen sind zu den „Nomina conservanda" die „Nomina oblita" gekommen. Bei einem ausreichend freien Spiel dieser Sicherheitsventile — und darauf kommt es an — dürften die Gespenster der Vergangenheit gebannt sein und die Klagen nach dieser Richtung verstummen. Offene Türen tritt man nicht ein. Daher ist hierzu eigentlich nichts mehr zu sagen.

Schmerzen bestehen heute vor allem da, wo sich die Verzahnung zwischen Nomenklatur und Feinsystematik auswirkt. Gerade hier ist gegenseitiges Verständnis dringend. Daher waren Herrn Albertis Bemühungen so wichtig, und ich konnte mich seiner und der Kongreßleitung Aufforderung zur Mitarbeit nicht versagen. Die zahlreichen Anmeldungen zeigen, wie aktuell dies Symposion ist.

Es sei hiemit eröffnet und begonnen mit einem herzlichen Dank an die Leitung dieses Museums und vor allem Herrn Dr. Beier, dem dies Symposion wohl mehr Last gebracht hat als andere.

Das Wesen des Symposions ist der engere geschlossene Kreis, der sich aus den zur Mitwirkung Aufgeforderten, die diese Aufforderung angenommen haben, zusammensetzt. Ein großer Kreis von Zuhörern ist eine Ehre für das Symposion. Bei Zeitmangel sprechen aber nur die eigentlichen Mitglieder in Rede und Gegenrede oder bei — was hoffentlich nicht nötig wird — quantitativer Feststellung des Stimmungsbildes. Solche Einschränkung liegt im Wesen des Symposions, das eben keine Sektion des Kongresses ist.

Die vorgesehenen Diskussionszeiten ermöglichen wahrscheinlich keinen völligen Ausgleich der offenbar befruchtend gegensätzlichen Meinungen, wie denn überhaupt die moderne Diskussion in Wort und Schrift, bestehend aus einmaliger Gegenrede und Schlußwort, nur noch ein Rudiment der ehemaligen Disputationen ist. Aber die Vorträge und gewisse auf dem Wege der Diskussion extrahierte Erläuterungen, geben hoffentlich die Unterlage zu dem Wichtigsten, der gründlichen Aussprache außerhalb unseres Saales bei Wein und Kaffee. Sie kann mehr als Aufsätze fördern.

Zur Tagesordnung: Wie wäre es, wenn uns Herr Alberti, der die Hauptarbeit für dies Symposion geleistet hat, erst einmal die Ziele selbst sagt, die ihm vorgeschwebt haben, und zwar zunächst außerhalb der Diskussion, wir dann die Wünsche aus der Versammlung besprechen und dann Herr Kollege Kraus die Leitung des wissenschaftlichen Teiles übernimmt, weil er kraft seines guten Englisch unseren ausländischen Kollegen den Inhalt der deutschen Vorträge im Kurzreferat nahebringen kann?

ZUR FRAGE DER MUTMASSLICHEN TYPEN UND DER UNRICHTIGEN ORIGINALDIAGNOSEN UND DARAUF BASIERENDEN UND UNBEGRÜNDETEN NOMENKLATURÄNDERUNGEN

Dr. VLADIMÍR BALTHASAR

Wir kennen unzählige Fälle, in denen es auf Grund des nachträglichen Studiums des Typenmaterials einer Art durch einen späteren Forscher zu nomenklatorischen Änderungen, welche öfters nicht nur die studierte Art selbst, sondern auch die nahverwandten Arten betroffen haben, gekommen ist. In vielen Fällen waren diese Änderungen ebenso vom rein nomenklatorischen, wie auch vom taxonomischen Standpunkte aus vollkommen berechtigt und haben zur Klärung des Begriffes der betreffenden Art viel beigetragen. Solche Änderungen waren unbedingt nötig, und es wird sich kaum jemand finden, der dagegen etwas einzuwenden hätte. Leider kennen wir aber mindestens ebenso viele Fälle, in welchen völlig unbegründete Eingriffe in die Nomenklatur durchgeführt worden sind, welche vom taxonomischen Standpunkte aus unbegründet waren und deshalb nicht nur höchst unerwünscht, sondern direkt verwerflich und unannehmbar sind. Und eben mit dieser Gruppe der Namensänderungen will ich mich diesmal befassen.

Zuerst müssen wir unsere Aufmerksamkeit den qualitativen Eigenschaften eines Typus widmen. Wir können die Typen in gewisse Kategorien einteilen und es wäre unrichtig, darin nur eine unnütze Spielerei mit Begriffen zu erblicken. Im Gegenteil, es wäre höchst notwendig, wenn sich jene Forscher, welche eben die Validität einer Art oder die Richtigkeit der Benennung jener Art auf Grund des Typenstudiums untersuchen, immer zuerst die Frage über die qualitativen Eigenschaften des Typus beantworten würden, bevor sie sich zu irgendwelchen Beschlüssen, die die Taxonomie oder Nomenklatur betreffen, entschließen.

Von dem erwähnten Standpunkte aus können wir die Typen folgendermaßen einteilen:

1. Absolut verläßliche Typen, also solche Belege, welche nicht nur das grellrote Zettelchen eines Holotypus, Allotypus oder Paratypus tragen, sondern welche auch wirklich Typen sind. Für die Typen gilt leider derselbe juristische Grundsatz, wie für den Vater, welcher „semper incertus est". Mit absoluter Bestimmtheit kann man sie nur in den Sammlungen der noch lebenden Autoren erwarten, vorausgesetzt, daß sich die Sammlung noch im Eigentum des betreffenden Wissenschaftlers befindet. Wenn aber ein Typus eines noch lebenden Autors sich außerhalb seiner Sammlung befindet, ist seine absolute Verläßlichkeit immer schon etwas gefährdet. Ich selbst kann drei in höchstem Maße belehrende Beispiele aus eigener Erfahrung anführen. Seiner Zeit habe ich für eine bekannte entomologische Firma eine außergewöhnlich reiche und viele neue, bisher unbekannte Arten der Scarabaeiden enthaltende Originalausbeute aus China bearbeitet. Ich habe allerdings nicht gewußt, daß ich immer nur einen Bruchteil dieses Materiales zum Studium erhielt. Der Eigentümer dieses Geschäftes schickte mir nur einen Teil der Tiere, die er nach seiner laienhaften Ansicht für eine Art hielt, die übrigen hat er zurückbehalten und nach der Rücksendung der determinierten Käfer bezettelt. Wenn es sich um eine neue Art handelte, deren Typen ich ihm retournierte, hat er die bei ihm gebliebenen und seiner Ansicht nach gleichartigen Individuen ganz ruhig mit Typenzettelchen versehen und als Typen (Paratypen) an die Interessenten versendet. Auf diese Weise existiert nun in verschiedenen Sammlungen eine beträchtliche Anzahl von meinen „Typen", die ich nie gesehen habe und von denen höchstwahrscheinlich eine bedeutende Anzahl überhaupt nicht jene Art vorstellt, die ich beschrieben habe. Im zweiten Fall handelt es sich ausgesprochen um „Typen-Apokryphe". Einer von meinen Kollegen synonymisierte seinerzeit eine von meinen Choeridium-

Arten mit einer älteren, von Preudhomme beschriebenen Art und berief sich dabei darauf, daß er im British Museum angeblich eine Reihe von Paratypen dieser meiner Art studieren konnte. Allerdings habe ich diese Art nach einem einzigen Individuum beschrieben, dieses befindet sich noch immer in meiner Sammlung und seit jener Zeit habe ich kein anderes Exemplar mehr gesehen. Und schließlich der dritte Fall, welcher diesmal nicht die Scarabaeiden, sondern Hymenopteren, Spheciden, betrifft und sogar etwas spaßhaft klingt. In einer Austauschsendung, welche nach gegenseitiger Vereinbarung auch eine Reihe von Paratypen enthalten sollte, entdeckte ich auch einen „Paratypus“ meiner eigenen Art, welche ich nach zwei einzigen Exemplaren meiner Sammlung beschrieben habe. Das, was ich anführte, genügt wohl zum Beweis, daß nicht einmal die „Typen“ der noch lebenden Autoren immer vertrauenswürdig sind.

2. In hohem Maße verläßlich sind die Typen der jüngeren, aber schon verstorbenen Autoren, deren Sammlungen sich in großen, wissenschaftlich verwalteten Museen befinden. Aus mehreren Gründen sind sie aber weniger verläßlich als die Typen der ersten Kategorie. In allen Museen der Welt, auch in jenen, die am sorgfältigsten verwaltet werden, wird leider von Zeit zu Zeit gestohlen. Und die Übeltäter rekrutieren sich nicht selten aus den Reihen der sonst ehrwürdigen Kenner. Für einen solchen Kleptomanen ist es eine Kleinigkeit, den ursprünglichen Typus durch ein Individuum einer ähnlich aussehenden Art zu ersetzen und dabei die Zettelchen und die Typenbezeichnung zu tauschen. — Und ebenfalls in allen Sammlungen der Welt sind die Insekten durch den Fraß der Anthrenen-Larven bedroht. Diese Larven sind manchmal sehr wählerisch. Der verantwortliche Desinfektor kann sehr leicht auf die unglückliche Idee kommen, sich dadurch vor den Vorwürfen seines Chefs zu schützen, daß er einen ähnlichen Ausweg wählt, wie der stehlende Entomophile.

3. Einen noch ziemlich hohen Grad der Verläßlichkeit besitzen die Typen der in den Museen aufbewahrten Sammlungen der Entomologen, deren wissenschaftliche Tätigkeit in die zweite Hälfte des vorigen Jahrhunderts fällt. Diese Autoren haben nämlich schon in den meisten Fällen die einzelnen Belegstücke ausdrücklich als Typus bezeichnet, und diese Typi sind deshalb von den übrigen Individuen derselben oder vermutlich derselben Art leicht zu unterscheiden. Es liegt in der Sache selbst, daß, je älter die Sammlung ist, umso weniger verläßlich die in ihr enthaltenen Typen sind.

4. Im allgemeinen als unverläßlich muß man die Typen jener Sammlungen bezeichnen, die in der zweiten Hälfte des 18. Jahrhunderts und in den ersten Dezennien des 19. Jahrhunderts entstanden sind. Die damaligen Autoren, und darunter auch die führenden, haben die Typen überhaupt nicht bezeichnet, man findet bloß eine kleinere oder zahlreichere Reihe von Individuen, von denen das erste die Etikette mit dem Namen der Art trägt, deren Autor als „mihi“ angeführt ist. Es kommt sehr oft vor, daß in dieser Reihe von sogenannten Typen sich zwei oder mehrere verschiedene Arten befinden, da die Kriterien einer Art damals bei weitem nicht so streng waren und weil die damaligen Entomologen sich primitiverer optischer Apparaturen bedienen mußten. Es kann aber sehr oft passieren, daß nicht einmal das einzige Individuum der vorhandenen Serie den Typus vorstellt, weil dieser während der langen Jahre vernichtet wurde oder einfach verloren gegangen ist. Diese alten Sammlungen haben oft eine sehr bunte und bewegte Geschichte hinter sich. Sie wurden oft mehrmals umgeordnet und dabei konnte es zu den verschiedensten Verwechslungen und ungewollten Vertauschungen kommen.

Es ist unbedingt notwendig, daß jene Autoren, die auf Grund des Studiums des typischen Exemplares irgend einer Art sich zu taxonomischen und daraus resultierenden nomenklatorischen Änderungen genötigt fühlen, vorerst alle Umstände, die ich angeführt habe, sehr sorgfältig prüfen. Es wäre ersprießlich, wenn ihnen die ganze Geschichte der Sammlung, besonders die Verläßlichkeit der bisherigen Aufbewahrung,

gut bekannt wäre. Jedenfalls ist es aber empfehlenswert, von irgendwelchen Änderungen des Inhaltsbegriffes der Art und deren Benennung abzusehen, wenn solche Änderungen auf Grund eines mutmaßlichen Typus dieser uralten Sammlungen durchgeführt werden sollten. Wenn das Studium dieser alten „Typen" unsere kontinuierliche Anschauung über eine Art bestätigt, dann gelangt die Überprüfung zu einem positiven Resultat und ist in diesem Sinne verwendbar. Im gegenteiligen Falle ist aber das Resultat negativ und dürfte zu keinen Änderungen benützt werden. Leider sind wir allzuoft Zeugen davon, daß eben solche negative Resultate zu weitgehenden nomenklatorischen Änderungen benützt werden und daß viele Autoren glauben zu ihrem eigenen Ruhm außergewöhnlich beigetragen zu haben, wenn sie sich um die Vermehrung der chaotischen Verhältnisse in der Nomenklatur auf diese Weise verdient machen.

Es ist nun unbedingt nötig, auch den Originalbeschreibungen (Original-Diagnosen) unsere Aufmerksamkeit zu widmen und die gegenseitigen Beziehungen zwischen ihnen und den Typen zu prüfen. Die Originalbeschreibung besitzt gegenüber dem Typus einen großen Vorteil. Sie ist unveränderlich, unvertauschbar und kann durch keine Handhabung vernichtet werden. Demgegenüber ist sie aber subjektiv, wogegen der Typus ein Objekt ist. Sie kann mit verschiedenen Beobachtungsfehlern und mit verschiedenen Fehlschlüssen, soweit sie die Vergleichung mit anderen, schon früher bekannten Arten der Gattung, ja sogar insofern sie die Einreihung in die betreffende Gattung anbelangt, belastet sein. Daraus ergibt sich die Notwendigkeit, daß wir — bevor wir eine den taxonomischen oder nomenklatorischen Zustand ändernde Entscheidung fällen — auch den Autor der Beschreibung einer Kritik unterziehen. Der Originaldiagnose eines erfahrenen und sorgfältigen Spezialisten werden wir gewiß größeres Vertrauen schenken, als der Diagnose eines in dem betreffenden Spezialfach nicht genug erfahrenen Autors, oder eines Autors, den wir schon wiederholt beim nachlässigen Vorgehen ertappt haben.

Es ist sehr bedauernswert, daß wir uns bei den Arten, welche in der ersten Periode nach der XII. Ausgabe der „Systema naturae" fixiert wurden (ungefähr bis zum Jahre 1820 bis 1830), weder auf die Typen, noch auf die Beschreibungen verlassen können. Die Diagnosen sind allzu kurz, manchmal ein- bis zweizeilig und können heute auf eine ganze Reihe von verwandten und habituell ähnlichen Arten bezogen werden. Die Typen, wie wir schon nachgewiesen haben, sind unverläßlich, wenn sie überhaupt heute noch vorhanden sind. Deshalb muß jede nomenklatorische Manipulation mit diesen Arten direkt oder indirekt zur Erhöhung der chaotischen Zustände in der Nomenklatur führen. Alle „tiefsinnigen" Erwägungen und alle Entscheidungen vom „grünen Tisch" aus schädigen nur den guten Ruf der modernen Systematik in den Augen der übrigen Zoologen. In diesen Fällen bleibt uns nur ein einziger Weg — und das ist der Weg der Kontinuität — sowohl im Begriffe der betreffenden Art, als auch in deren Benennung. Es ist unbestreitbar, daß die Bewahrung des Kontinuitätsprinzipes in solchen Fällen auch am besten dem ethischen Aspekt der Priorität entspricht. Man muß doch annehmen, daß jene Benennung und jene Auffassung der Art, die sich durch lange Jahrzehnte in den Arbeiten der hervorragenden Kenner, deren wissenschaftliche Tätigkeit nicht selten direkt an die Arbeiten der alten Autoren der Arten anknüpft und durch direkte Erfahrung gestärkt wird, am wahrscheinlichsten der objektiven Wahrheit entspricht.

Es könnte eingewendet werden, daß auch die Diagnosen einiger ganz moderner Autoren sehr wortkarg sind und uns eigentlich auch keine verläßliche Stütze bieten. Diese Tatsache ist leider unbestreitbar, jedoch handelt es sich um Ausnahmsfälle. Außerdem finden wir in solchen Fällen die beste Hilfe im direkten Studium der Typen, welche, wie schon gesagt wurde, je moderner, desto verläßlicher sind. Wenn die wortkarge Definition eines solchen bequemen Autors nicht im direkten Widerspruch mit den Merkmalen des Typus steht, erscheint uns eine solche Art vollkommen klar

definiert und wir können sie durch eine Redeskription nachträglich eindeutiger definieren, ohne an ihrer Definition oder Benennung etwas bedeutenderes ändern zu müssen.

Nun kommen wir aber zu einem speziellen Fall, welchem leider jeder wissenschaftlich arbeitende Taxonom oft begegnet. Solche Fälle bilden am häufigsten die eigentliche Ursache der taxonomischen und damit verbundenen nomenklatorischen Streitigkeiten und verursachen, daß auch bei neuzeitlichen Arten ein nomenklatorisches Chaos entstehen kann. Auf einer Seite steht eine ausführliche Originalbeschreibung eines schon verstorbenen, angesehenen Autors, eines bekannten Spezialisten, zu dessen Lebensarbeit wir das größte Vertrauen haben. Auf der anderen Seite steht der dazugehörende Typus in seiner Sammlung, welche nach dem Ableben des Autors in einem Museum aufbewahrt wird. Beim Studium dieses Typus konstatieren wir aber zu unserer größten Überraschung, daß der Typus in mancher Beziehung und in vielen wichtigen Merkmalen der Originaldiagnose nicht entspricht. Aus dieser Diskrepanz zwischen der Diagnose und dem Typus haben schon viele spätere Autoren falsche und unstatthafte Folgerungen deduziert, welche taxonomisch oder nomenklatorisch unannehmbar sind. Ich könnte an dieser Stelle viele sehr belehrende Beispiele anführen, welche auf solche Fälle klares Licht werfen dürften. Ich bin jedoch überzeugt, daß jeder erfahrene Entomologe mehrere Beispiele aus eigener Praxis kennt und daß es zeitraubend wäre, solche hier anführen zu wollen.

An solchen Beispielen könnte man mühelos demonstrieren, welche Folgen der absolute Gegensatz zwischen der Originalbeschreibung und zwischen dem Typus auslösen kann. Einerseits also richtige Originaldiagnose und falscher Typus, andererseits eine ausgesprochene und daher ungültige „descriptio falsa“ und wirklicher Typus. Im ersten Fall muß man die beschriebene Art, deren Autor vollkommen vertrauenswürdig ist, solange für eine existierende Art betrachten, bis uns jahrelange Erfahrungen nicht von einem Gegensatz überzeugen werden. Den Typus dagegen muß man für eine Fälschung erklären, unbeachtet, ob es sich um eine schon bekannte oder bisher unbekannte Art handelt. Im zweiten Fall nehmen wir an, daß der Typus verläßlich ist, er trägt aber ein „nomen nudum“, die Art ist daher unbeschrieben, weil die „descriptio falsa“ ungültig ist. Die Entscheidung für diese zweite Möglichkeit ist aber immer gewissermaßen gewagt, weil die absolute Verläßlichkeit des Typus — wie wir schon bewiesen haben — eigentlich sehr beschränkt erscheint.

Ich möchte noch bemerken, was als eine „descriptio falsa“ zu betrachten ist. Die wichtigste Voraussetzung zur Feststellung einer „descriptio falsa“ ist die Existenz eines denkbar verläßlichsten Typus. Ohne Konfrontation mit solchem Typus kann man keine Originaldiagnose für falsch erklären. Die unrichtigen Angaben müssen sich auf absolute und nicht relative Merkmale beziehen, also auf solche Merkmale, deren wörtliche Beschreibung sowie von dem subjektiven Eindruck des ursprünglichen Autors, so auch von der subjektiven Anschauung des revidierenden Fachmann unabhängig ist. Absolute Fehlerhaftigkeit muß sich außerdem auf solche Merkmale beziehen, von denen die einwandfreie Identifikation der Art abhängig ist, also nicht auf Nebenmerkmale oder sogar auf eventuelle falsche Patriaangaben, unrichtige ökologische Angaben und ähnliches.

Mit dem, was ich hier gesagt habe, erstrebte ich eigentlich, die Lösung eines und desselben Problems von zwei verschiedenen Seiten zu erreichen. Erstens wollte ich eine ernste Mahnung an jene Entomologen richten, die das Studium und die Revision der alten Typen fast zum Selbstzweck erhoben haben und die sich einbilden, daß sie dadurch der Taxonomie einen großen Dienst erweisen. Sie scheinen besonders stolz darauf zu sein, wenn sie möglichst viele Lectotypen und Neotypen statuieren können, auf deren Grund sie ganz überflüssige und verwirrende Nomenklaturänderungen im Namen des Prioritätsprinzipes durchführen können. Diese Tätigkeit trägt sehr viel zur Abneigung

der großen Mehrheit der Entomologen zum Prioritätsprinzip, resp. zu dessen Durchsetzung um jeden Preis, welches Verfahren zum Verlust der anscheinend erstrebten Stabilität der Nomenklatur führt und chaotische, ruhige und konstruktive wissenschaftliche Arbeit bedrohende Verhältnisse schafft. Ich wäre im höchsten Maße zufrieden, wenn mein Vortrag zum bedachtsameren Vorgehen beim Studium der Typen und zur erhöhten Enthaltsamkeit bei den eventuellen taxonomischen und nomenklatorischen Änderungen führen würde.

Zweitens aber wollte ich die internationalen Instanzen, denen die Regelung und in erster Linie die Genesung der Verhältnisse in der zoologischen Nomenklatur obliegt, auf diese spezielle Frage aufmerksam machen, da auch sie mit der neuen Auffassung der „nomina oblita" eng zusammenhängt und dieser Auffassung widerstrebende Tendenzen unterstützt. Denn eben die unkritischen Typen-Verehrer haben viel dazu beigetragen, daß viele vergessene Namen ganz überflüssig zum neuen Leben erweckt wurden und ebensoviele eingelebte, durch Kontinuität stabilisierte und allgemein bekannte und benützte Gattungs- und Artnamen im Abgrund der Synonyme verschwinden mußten. Es wäre daher sehr wünschenswert, alle solche unkritische Änderungen, die der kontinuierlichen Auffassung einer Art und deren durch die Kontinuität stabilisierten Benennung widerstreben, als eine gegen die internationalen Nomenklaturregeln verstoßende Handlung ausdrücklich zu bezeichnen.

SUR LA CONFUSION DES DÉSINENCES UM ET ON DANS LES NOMS DE GENRE

PIERRE BONNET

> Mais si sur quelque article existe l'injustice
> Un manque de logique ou qu'une erreur se glisse
> Avec force vigueur employez-vous alors
> A corriger la règle et redresser les torts
>
> P. Bonnet, De la Nomenclature, essai poétique

J'ai déjà à deux reprises[1] signalé l'extrême confusion qui régnait dans l'orthographe des termes génériques se terminant par *um* et *on*, car un très grand nombre de ces termes sont écrits avec l'une et l'autre désinence (*Theridion* et *Theridium*, *Zodarion* et *Zodarium*, *Apion* et *Apium*, *Myrmecion* et *Myrmecium* etc.).

Je crois cependant devoir revenir une troisième fois sur la question qui est d'une extrême importance, pensant que de nonveaux arguments arriveront à convaincre les naturalistes de la nécessité d'une graphie régulière de ces noms.

Cette double graphie vient de ce que, le plus souvent, des auteurs écrivent un de ces termes d'une façon erronée et que, par la suite, un autre auteur, mieux renseigné, a corrigé la faute initiale. A partir de ce moment là, les deux orthographes sont utilisées, l'une parce que l'on admet la graphie originale fautive, l'autre parce que l'on préfère l'écriture correcte. Parfois, cela provient aussi de ce que certains auteurs, par simple

[1] Difficultés de Nomenclature chez les Aranéides. XVII: Il faut écrire Theridium. *Bul. Soc. hist. nat. Toulouse*, 92 (1957), pp. 231—239. Homogenity and correction of scientific terms in zoological nomenclature. *Bul. Zool. Nomencl.*, vol. 15 (1958), pp. 645—661.

souci d'homogénéité corrigent des noms corrects sans se rendre compte qu'ils n'ont pas même origine. De toutes façons, on arrive à avoir des noms écrits de deux façons différentes et rien n'est aussi insupportable en Nomenclature.

C'est ainsi, en reprenant les exemples déjà cités, que nous avons:

terme initial fautif	terme rectifié
Theridion	*Theridium*
Zodarion	*Zodarium*
terme initial correct	terme faussement corrigé
Myrmecium	*Myrmecion*
Apion	*Apium*

Il importe donc de savoir quels sont les noms qui doivent s'écrire correctement avec *um* et ceux qui doivent se terminer normalement par *on*. La question est pourtant assez simple. En latin,

um (genitif *i*) est la désinence du neutre (*templum*, *bellum*, *caecum*, *ornamentum*, *musaeum*, etc. . .) et, évidemment, il n'y a pas de noms de personne dans cette catégorie.

on (génitif *onis*) est une désinence du masculin (*aeon*, *canon*, *ancon*, *andron*, *eon*, *lycon*, etc. . .) et se rencontre dans de nombreux noms de personne (*Arion*, *Philemon*, *Samson*, *Sarpedon*, *Simeon*, *Platon*, *Orion*, etc. . .) ainsi que les termes composés avec *odon*, dent (*Mastodon*, *Iguanodon*, . . .).

Ces désinences *um* et *on* correspondent aux désinences grecques ον et ων:

	latin	grec	
masculin:	*on*	ων	(avec un oméga)
neutre:	*um*	ον	(avec un omicron)

Ainsi, tous les mots grecs qui se terminent par ων doivent se latiniser en *on* (et être du genre masculin) et tous ceux finissant par ον doivent se latiniser en *um* (et être du genre neutre). Cette règle était celle des Romains eux-mêmes dans la translittération des mots grecs.

Il n'y a pas de raison pour que nous n'appliquions pas en Nomenclature, cette règle si simple pour les noms génériques. Il y a, au contraire, de bonnes raisons d'intelligence, de logique, de simplicité pratique et même d'harmonie graphique pour qu'il en soit ainsi.

Il est, en effet, évident que l'on doit toujours translittérer les mêmes lettres d'une langue par les mêmes lettres d'une autre langue, et en ce qui nous occupe ici, si

Χειραχάνθιον	se transcrit par	*Chiracanthium*
Θηριδιον	doit se transcrire par	*Theridium*

Et je pense que l'on doit s'indigner d'apprendre que sur le terme correct *Myrmecium* on a formé les mots *Megamyrmaekion* et *Paramyrmecion*! (Pourquoi pas *Promyrmetion* on *Pseudomyrmessium*, s'il avait plu à quelqu'un d'écrire ainsi ces noms?)

En Nomenclature zoologique, il doit exister plusieurs centaines de termes génériques finissant par *on* et par *um*. Il est, à mon avis, d'un intérêt considérable qu'ils soient tous soumis à cette règle et comme beaucoup doivent avoir les deux graphies, il importe pour chacun d'eux de n'utiliser désormais que la bonne. En conséquence:

si les noms sont du neutre, ils doivent se terminer par *um*
si les noms sont du masculin, ils doivent se terminer par *on*

et corrélativement

les noms se terminant par *on* doivent être du masculin
les noms se terminant par *um* doivent être du neutre.

A partir de ce moment chacun verra clair en cette question, saura la graphie correcte qu'il convient d'employer et l'accord convenable qu'il faut faire. On ne verra plus de *Susarion neglectum*, *d'Apium violaceus*, ni de *Theridion denticulatum*, qui sont des solécismes, alors qu'il faut dire et écrire *Susarion neglectus*, *Apion violaceus* et *Theridium denticulatum*.

On m'a objecté que l'application de cette règle entraînerait des modifications trop importantes pour des quantités de noms. Je ne le crois pas, car là encore je peux donner des chiffres précis et très significatifs. En effet, dans le groupe des Aranéides, nous avons 82 noms de genre finissant par *on* ou par *um*:

46 termes en *um* (dont 11 en synonymie) du genre neutre,
19 termes en *on* (dont 7 en synonymie) du genre masculin
(par conséquent 65 noms corrects, sur 82),

et 17 termes en *on* (dont 3 en synonymies) qui, étant du neutre, devraient se terminer par *um* et sont, de ce fait, incorrects.

Sur ces 17 noms, réduits à 14 (en négligeaut les 3 synonymes) il y en a 4 pour lesquels la rectification a déjà était faite: *Theridion* → *Theridium*; *Zodarion* → *Zodarium*; *Rhion* → *Rhium*; *Megamyrmekion* → *Megamyrmecium*, et là il était pour moi normal d'adopter le terme correct rectifié en *um*. Il restait donc dix noms en *on* qui devaient être en *um*; je n'ai pas hésité à faire ces rectifications par application de la règle envisagée, surtout que ces dix noms étaient encore jusqu'ici peu employés.

Je suis en droit de penser que dans tous les groupes zoologiques il doit en être de même et dans les mêmes proportions; de sorte que l'application de cette règle ne peut entraîner de grosses perturbations comme on l'a objecté et l'avantage considérable qu'elle présente vaut bien qu'elle soit intègralement appliquée.

Un autre inconvénient d'avoir des noms incorrects, c'est que si un de ces termes est utilisé pour former un nom de groupe, on risque fort de le mal former. C'est ce qu'a fait normalement W. Crome dans ses *Arachnida* de Exkursionsfauna von Deutschland en formant le nom de famille *Theridionidae* (p. 310); il ne se serait pas ainsi trompé s'il avait écrit *Theridium*.

Enfin, il est bien certain que si l'on ne se soumet pas uniformément à cette réglementation, on n'arrivera jamais à s'entendre en Nomenclature; car si les partisans de la graphie originale erronée ne veulent pas modifier leur point de vue, peuvent-ils décemment nous reprocher d'écrire des noms corrects et nous obliger à faire des fautes d'orthographe!

QUELQUES RÈGLES INTERNATIONALES MAL OU PAS APPLIQUÉES

S. G. KIRIAKOFF, Gand

Le Code de la nomenclature zoologique tel qu'il a été discuté au dernier Congrès (Londres 1958) n'est certes pas le dernier mot en la matière. Son objet, défini comme étant «d'établir les bases d'un ensemble stable et universel de noms pour les groupes taxonomiques des animaux» (préambule au texte français du Code, *Bull. Z. N.*, vol. 14, 1957/58, p. 381), montre clairement qu'il s'agit d'une œuvre de longue haleine et susceptible d'être modifiée tout au long de son élaboration.

Cela n'empêche que les différentes «règles» qui composent ce code, soient le résultat d'un labeur chaque fois prolongé, parfois pénible, où tous les points de vue ont été examinés. Ce résultat, pour être officiellement valable, doit obtenir l'adhésion de la majorité des zoologistes qui se donnent la peine d'assister aux Congrès. L'acceptation d'un texte se fait d'après un règlement assez rigide, mais du moins peut-on dire que, dans les conditions actuelles, et même tenant compte de certains côtés négatifs du règlement sur lesquels nous ne nous proposons pas d'insister aujourd'hui — le texte d'une «règle» correspond à l'opinion de la majorité. Or, dans le monde moderne c'est l'opinion de la majorité qui compte. Une minorité même importante doit se soumettre loyalement à cette opinion, et cela aussi longtemps qu'une autre majorité ne se forme pas, cette fois pour modifier la disposition légale ou toute autre en question.

Une loi doit être observée par tous. S'il s'agit d'une loi proprement dite, il existe généralement un dispositif destiné à la faire respecter par l'emploi de sanctions contre les personnes coupables de ne pas le faire. Une «loi» de la nature des Règles de la nomenclature scientifique ne peut évidemment prétendre à pareille protection. Elle dépend entièrement de la bonne volonté des intéressés. E. G. Linsley et R. L. Usinger (Linnaeus and the Development of the International Code of Zoological Nomenclature, *Syst. Zool.*, vol. 8 (2), 1959, p. 39—47) le disent excellemment: «Civil laws are backed by police enforcement; the laws of nomenclature depend upon voluntary support of zoologist».

Il est clair que toute «règle», étant le produit d'un compromis, compte des adversaires. Ce n'est que sur le principe que tout le monde est d'accord. Qu'on songe cependant à deux aspects de la question:

a) à tout moment donné, le Code forme un *tout*, un ensemble, où chaque chapitre, chaque article, chaque paragraphe et même chaque mot a la *même valeur* théorique, ayant été approuvé par la même procédure;

b) à tout moment donné (avec les restrictions d'ordre formel résultant de la procédure en vigueur), toute disposition du code peut être changée. Il n'appartient cependant ni à un zoologiste isolé, ni à un groupe de zoologistes, de quelle valeur scientifique qu'il soit, de modifier une ligne, un mot ou une lettre du texte officiel. Le changement, aussi minime qu'il soit, ne peut se faire que suivant la procédure officiellement établie.

Si donc l'on accepte les règles de la nomenclature zoologique, on doit les accepter toutes. Ceux qui font une sélection en rejetant les règles qui ne leur conviennent pas, font au but du Code — qui est la stabilité de la nomenclature — autant de tort que ceux qui ne veulent reconnaître aucune règlementation en la matière. Nous ne savons du reste pas s'il existe encore un spécimen de cette dernière catégorie de zoologistes.

Il est d'autant plus pénible de devoir constater que certains articles du Code semblent faire un objet favori d'infractions. Si nous connaissons les raisons de cet état de choses en ce qui concerne certains de ces articles, dans un cas au moins on se trouve devant une situation réellement incompréhensible.

Nous allons examiner ci-dessous trois cas, et nous commencerons précisément par celui qui ne paraît pas être provoqué par des considérations théoriques ou sentimentales.

1° Les noms subspécifiques.

Le Code dit au sujet des noms subspécifiques ce qui suit (Art. 15, section 7[a]):

«La désignation d'une sous-espèce doit être constituée (1) par le binôme auquel appartient le nom subspécifique suivi (2) du nom subspécifique sans intercalation d'aucun signe de ponctuation. Exemple: *Rana esculenta marmorata* Hallowell, et non *Rana esculenta* (*marmorata*), ni *Rana marmorata* Hallowell, ni *Rana esculenta*, subsp. *marmorata* Hallowell».

Il nous paraît que l'énumération des désignations n'est pas limitative, et que ces désignations y figurent à titre d'exemple seulement. On peu y ajouter d'autres,

aussi fautives, comme ssp. *marmorata* Hallowell, car cette désignation ne contient pas le binôme mentionné dans l'alinéa (1) ci-dessus, mais seulement le nom subspécifique qui est, en outre, précédé par une abréviation du terme subspecies non prévu dans les dispositions en vigueur.

Malgré les termes précis de ces dispositions, les infractions à l'article 15, section 7 (a) sont tellement nombreuses que chacun des zoologistes ici présents pourra sans doute en citer quelques-unes. Nous voudrions cependant donner ici deux-trois exemples. H. Hedicke dans sa contribution «Heteroptera» in Die Tierwelt Mitteleuropas, IV/3, parle (p. X, 107) de (*Carpocoris*) *pudicus* Pd. et de sa subsp. *fuscispina* Blch. W. Forster, in Die Schmetterlinge Mitteleuropas, vol. II, p. 11, distingue «die typische ssp. *bryoniae* O.» et les ssp. *flavescens* F. Wagner et ssp. *neobryoniae* Shelj. Enfin, R. Paulian, dans le volume Coléoptères Scarabéides, in Faune de France, vol. 63, 1959, dit (p. 39): «*L.* (*ucanus*) *tetraodon* est représenté en France continentale par la subsp. *provincialis* Colas . . .» Ce sont évidemment des exemples choisis au hasard parmi un grand nombre de cas semblables. Nous avons mentionné trois ouvrages de grande diffusion, destinés en premier lieu à aider les amateurs, et à notre sens ce fait aggrave considérablement le cas. En effet, les auteurs de ces ouvrages sont des spécialistes de premier plan, possédant une autorité considérable, d'ailleurs très méritée, et la plupart des modestes amateurs qui utilisent les manuels en question se garderont sans doute bien de contredire les maîtres.

Le respect de l'article 15 s'impose donc, en dehors des considérations d'ordre général émises plus haut, aussi tout particulièrement à cause de son importance pratique.

2° Accord des noms génériques et spécifiques.

Les dispositions du Code stipulent (Art. 28, section 13 [1]) que le nom spécifique peut être, entre autres, «Un adjectif au nominatif singulier du même genre grammatical que le nom générique avec lequel il forme à un moment un binôme ou un trinôme, c'est à dire il doit être accordé grammaticalement avec son nom générique . . .» Les noms subspécifiques sont assimilés ici aux noms spécifiques.

Les infractions à la disposition ci-dessus peuvent être rangées dans deux groupes:

a) celles résultant de l'ignorance, p. ex. *Spilosoma lutea* au lieu de *Spilosoma luteum*;
b) celles commises intentionnellement.

Ce sont ces dernières qu'il faut combattre.

L'argument invoqué le plus souvent par les personnes en défaut est qu'un nom spécifique (ou subspécifique) doit conserver sa forme originale, car il constitue en quelque sorte un prénom fixé par l'état civil et ne pouvant plus être modifié.

Remarquons tout d'abord qu'il ne s'agit ici que d'adjectifs au nominatif du singulier, les noms spécifiques ou subspécifiques formés autrement restant invariables et donc en dehors de toute discussion.

Voici d'ailleurs des exemples:

Polyommatus hispana H. S. (in L. Lhomme, Catalogue des Lépidoptères de France et de Belgique, vol. I, p. 97).

Hesperia armoricanus Ob. (ibid., p. 108).

Quant au fond de l'argument ci-dessus, on peut y répondre que n'importe quel auteur qui décrit une nouvelle espèce ou sous-espèce et lui donne un nom spécifique formé d'un adjectif, accorde ce nom avec le nom générique, existant ou nouveau. Ce procédé nous paraît élémentaire, et du reste en accordant le nom spécifique avec un nouveau nom générique d'un genre grammatical différent, on ne fait en somme que respecter la volonté de l'auteur original qui, lui, avait bien fait l'accord.

Ensuite, lorsqu'il s'agit de «prénoms fixés par l'état civil», il ne s'agit au fond que de noms propres ou à la rigueur de substantifs. D'autre part, les noms scientifiques étant

sensés être latins, il convient de suivre l'usage romain à ce sujet. Or, chez les Romains le prénom personnel et même le nom de famille étaient bien accordés avec le sexe du porteur, p. ex. Caius Julius et Caia Julia.

Nous croyons donc que l'argumentation des adversaires de l'accord ne repose pas sur des fondements solides. Nous avons, en tous cas, le texte formel du Code lequel serait à suivre même si cette argumentation était irréfutable.

Il est peut-être utile d'attirer l'attention sur l'aspect «primaire» et même «barbare» des noms comme ceux cités ci-dessus.

3° Emploi d'initiales minuscules.

Ici encore, le Code est formel (Art. 28, section 14): «Emploi d'une initiale minuscule: tout nom spécifique, subspécifique et infra-spécifique doit être écrit avec une lettre initiale minuscule.»

Cette disposition est relativement récente et ne fait que sanctionner un usage devenu presqu'universel. Il n'y a guère qu'une partie d'auteurs français et belges qui persistent encore à employer une initiale majuscule lorsqu'il s'agit de noms spécifiques ou subspécifiques formés d'un nom de personne.

L'argument semble être ici d'ordre purement sentimentel: courtoisie envers la personne honorée par l'auteur du nom, et surtout maintien d'une tradition vieille de près de deux siècles.

Il y a peu de gens qui ne tiennent pas aux traditions, aussi le motif invoqué par les partisans de la majuscule nous paraît-il plus sympathique que dans le cas précédent. Il faut cependant se soumettre au texte officiel, qui exprime l'avis et sanctionne l'usage de la très grosse majorité. Nous faisons ici appel à la bonne volonté de nos collègues, dans le but de contribuer à une unité complète dans cet usage.

Notre exposé n'épuise pas la question. D'autres infractions que celles examinées ici sont commises de temps en temps. Très fréquente p. ex. est la non-observation de l'article 14, section 1 (b) qui exige qu'«un genre divisé en sous-genres doit toujours porter le même nom que le sous-genre ayant le plus ancien nom valable et qui, en conséquence, est appelé «sous-genre tautonyme». Or, de nombreux auteurs placent l'espèce type du genre à scinder dans un sous-genre portant un nom différent de celui du genre, le plus souvent par le préfixe «Eu-». Cette pratique doit, elle aussi, être sévèrement condamnée.

Et nous terminons cet exposé en soulignant encore la nécessité urgente d'abandonner tout déviationnisme en matière de la nomenclature zoologique. Que chacun songe au vieux dicton «Dura lex sed lex», et que chacun réalise que l'acceptation libre d'une règle renforce cette dernière beaucoup plus efficacement qu'une sanction.

DISCUSSION

G. BERNARDI: Je suis tout à fait d'accord avec Mr. Kiriakoff à propos des exemples précis qu'il a cités: il faut utiliser la minuscule pour écrire les noms d'espèces etc, etc . . . Je pense donc que beaucoup d'auteurs s'écartent sans aucune nécéssité et sans motifs valables des Règles. Je ne crois pas cependant qu'il faut poser en principe absolu l'obéissance à celles-ci.

En effet dans quelques cas les Règles actuelles portent atteinte à la liberté du travail et des idées taxonomiques bien qu'elles affirment solennellement le contraire dans leur Préambule. Ainsi la nouvelle recommandation de ne pas citer un terme autre que le sous-genre entre les noms générique et spécifique est en pratique une condamnation de toutes les tentatives d'exprimer la vicariance géographique supraspécifique dont l'importance en matière d'évolution n'est plus à démontrer depuis Mayr (1942).

Il est légitime dans de tels cas, à mon avis, de ne pas tenir compte des dispositions des Règles.

LES RÈGLES INTERNATIONALES DE LA NOMENCLATURE ZOOLOGIQUE ET LA TAXONOMIE ÉVOLUTIVE

G. BERNARDI

Il y a conflit entre la notation traditionnelle des Règles et les notations recherchées en taxonomie évolutive[1] (= Nouvelle Systematique) parce que:

1) les Règles révisées de Londres (1958) continuent à envisager les catégories taxonomiques seulement comme un moyen commode pour inventorier espèces et populations d'animaux au moyen du binôme ou du trinôme classiques.

2) les partisans de la taxonomie évolutive tentent d'utiliser ces catégories non seulement pour désigner un animal ou une population mais aussi pour exprimer les différentes étapes de l'évolution.

Il est ainsi urgent d'aborder au Symposium deux questions écartées des Règles et actuellement en pleine anarchie:

I. Incorporation dans la désignation scientifique des animaux de catégories supplémentaires.

a) d'une manière générale il faut éviter, à mon avis, sous prétexte d'adapter les Règles à la taxonomie évolutive, de proposer une notation obligatoire trop longue, indésirable pour certaines disciplines (zoologie appliquée etc.). Il suffit pour cela d'admettre désormais deux notations: une brêve et stable notation classique (binôme et trinôme des Règles) et une notation pour spécialistes plus complexe et peut être plus instable, à catégories supplémentaires toujours facultatives incorporées aux précédentes en fonction de la nature d'un travail (sous-genre, superspecies, exerge etc.)

Parmi ces catégories supplémentaires doit donc figurer le sous-genre dont il serait souhaitable, à mon avis, de recommander l'emploi afin de permettre l'utilisation de vastes genres et rendre à cette derniere catégorie sa fonction primitive dans la notation linnéénne.

b) il est essentiel d'unifier les initiatives individuelles de notation des nouvelles catégories taxonomiques des ornithologistes Amadon, Bemmel, Laubmann et des lépidoptéristes Kiriakoff, Lorkovic, Remington, Toxopeus et moi-même. On trouvera une étude critique de ces notations dans mon travail de 1958 (p. 232—240). Je crois qu'il est surtout utile d'adopter une notation unifiée pour deux étapes particulièrement importantes de l'évolution, ignorées des Règles:

A. Vicariance d'espèces monophylétiques (superspecies).

La minorité d'auteurs qui proposent d'exprimer ce phénomène au moyen d'une catégorie taxonomique incorporent tous cette catégorie entre les noms générique et spécifique: *Papilio U* [*machaon*] *indra* Reak. (Remington, 1948); *Dicrurus* [*hottentotus*] *montanus* (Kiriakoff et Lorkovic, 1958); *Hemignathus* (*lucidus*) *wilsoni* Roth (Amadon, 1947a et Bernardi, 1958).

Les Règles révisées s'opposent implicitement à toutes ces notations car elles recommandent que «pour éviter tout malentendu un synonyme ou un terme autre que le sous-genre ne devrait jamais être cité entre l'élément générique et l'élément spécifique

[1] Nous préferons employer en France cette expression de Boquet à la place de l'expression nouvelle systématique des auteurs de langue anglaise (cf. Boquet, 1953, p. 191—193 pour les motifs de ce choix).

d'un binôme». Je propose de négliger cette recommandation car elle «porte atteinte à la liberté des idées et du travail taxonomiques» et d'incorporer en tant que catégorie facultative la superspecies Mayr, 1931 = Artenkreis Rensch, 1929, avec la notation suivante pour une espèce d'une superspecies:

Colias (*electo*) *croceus* Fourcr.; *Chrysocarabus* (*auronitens*) *punctato-auratus* Germ.; c'est à dire Genre (superspecies) espèce.

Je me permets d'insister pour l'emploi des parenthèses au lieu des crochets proposés par Kiriakoff et Lorkovic (1958):

a) parce qu'elles ont été utilisées de cette manière dès 1947 par l'ornithologiste Amadon, puis en 1959 par le mammologiste Dandelot et seront donc mieux accueillies que les crochets par les zoologistes non entomologistes — b) parce que les parenthèses sont d'un emploi plus simple en typographie et seront donc plus facilement admises dans toutes les revues.

B. Affinités entre une partie des populations d'une espèce (exerge).

Il est courant en entomologie et dans quelques autres disciplines d'exprimer l'affinité que présentent certaines populations d'une espèce par l'emploi simultané de deux catégories intraspécifiques obligatoires: *Cicindela campestris corsicana* natio *saphirina* Guéné (Semenov Tian Sanskij, 1911); *Glossina morsitans submorsitans* race *ugandensis* Vanderplank, 1949; *Triturus cristatus carnifex* var. *albanicus* Dely, 1959; *Alcedo atthis hispidoides pelagica* Streseman (Laubmann, 1932).

Les Règles s'opposent à toutes ces notations. En effet selon les Règles révisées a) sauf s'il s'applique à une partie d'une population un nom qui désigne une entité caractéristique d'une région géographique doit être considéré comme un nom subspécifique — b) la désignation d'une sous-espèce doit être un trinôme. En d'autres termes les exemples ci-dessus doivent etre rectifiés de la manière suivante pour être conformes aux Règles: *Cicindela campestris saphirina* Guéné; *Glossina morsitans ugandensis* Vand., *Triturus cristatus albanicus* Dely; *Alcedo atthis pelagica* Stresm.

Comme la notation trinominale est largement adoptée par la majorité des zoologistes et afin d'éviter un conflit permanent avec les Règles je propose d'incorporer seulement comme catégorie facultative l'exerge de Verity, 1929 (= Formengruppe Laubmann = grex Toxopeus, 1930) avec la notation suivante pour une sous-espèce d'un exerge:

Melitaea athalia (*celadussa*) *nevadensis* Obth.; *Pyrgus malvae* (*malvoides*) *elegantior* Vrty; c'est à dire genre espèce (exerge) sous-espèce.

On notera que l'exerge ne doit pas être utilisé pour exprimer l'affinité immédiate d'un petit nombre de populations voisines très proches géographiquement mais seulement l'affinité plus générale d'un grand nombre de populations déjà bien distinctes et en principe largement répandues. Ainsi je ne crois pas que la notation ci-dessus s'applique à des populations telles que *Zygaena rhadamanthus grisea* var. *azurea* Burgeff et *Z. rhadamanthus grisea* var. *stygia* Burgeff, toutes deux volant dans les Alpes maritimes. La variation géographique de telles populations est mieux exprimée à mon avis au moyen d'une catégorie infrasubspécifique ainsi que sela sera montré dans le chapitre suivant.

II. Ampleur de la sous-espèce

On utilise actuellement en Zoologie (cf. Amadon, 1947b; Bernardi, 1958; Pimentel, 1958; Rand et Traylor, 1950; Edwards, 1954 etc.) des sous-espèces non comparables, correspondant a des étapes très différentes de l'évolution. Il s'agit a) de diverses sous-espèces «faibles» (sous-espèces dites de la différence de moyenne, des règles de 50 ou de 75% etc.) dont, au mieux, seule une partie des individus diffèrent des individus des autres sous-espèces — b) d'une sous-espèce «forte» basée sur la règle des 100% dont

tous les individus sont différents des individus des autres sous-espèces. Cette dernière sous-espèce est souvent utilisée en lépidoptérologie (cf. Warren, 1936; Higgins, 1941, 1955; van Son, 1949, 1955) avec une catégorie infrasubspécifique s'appliquant seulement à une partie d'une population mais employée également pour l'étude de la variation géographique et plus exactement pour l'étude de la variation géographique du polymorphisme de ces populations (morpha Semenov, 1906 = form Warren, 1936 = modification Higgins 1941).

Ainsi pour Warren (1936) qui utilise une sous-espèce «forte» l'*Erebia gorge* mph. *erynis* Esp. n'est pas une sous-espèce bien qu'elle représente la forme caractéristique d'*Erebia gorge* dans les Alpes maritimes et les Basses Alpes parce qu'elle n'élimine pas entièrement la forme nominative *gorge* de ces régions. Au contraire en termes de sous-espèce faible les *E. gorge* des Alpes maritimes et des Basses-Alpes seront isolés en une sous-espèce particulière (*E. gorge erynis* Esp.) dissimulant l'existence dans ces régions d'exemplaires identiques à ceux d'une autre sous-espèce (*E. gorge gorge* Hb.). Inversement selon Martin-Brown et Comstock (1952) qui utilisent une sous-espèce «faible» les *Heliconius charitonius* L. de Cuba et de l'Amérique centrale sont des sous-espèces distinctes parce qu'ils présentent une différence significative de la fréquence des individus a espaces clairs des ailes roussâtres au lieu de jaunes (seulement 8,7% chez les ♀ d'*H. ch. ramdseni* de Cuba au lieu de 61,6% à 65,4% chez les ♀ d'*H. ch. vasquezae* de l'Amérique centrale). En termes de sous-espèce «forte» ces *Heliconius* seraient réunis en une seule sous-espèce (*H. ch. ramdseni*) en désignant éventuellement les individus aussi bien centre-américains que cubains à espaces roussâtres sous le nom d'*H. ch. ramdseni* mph. *vasquezae* Brown et Comst.

Par contre des sous-espèces telles que l'*E. gorge albanica* Rebel ou qu'*H. ch. peruvianus* Feld. qui éliminent toute autre forme d'*E. gorge* ou d'*H. charitonius* de l'Albanie ou du Pérou seront des sous-espèces en termes de sous-espèces «forte» et, à fortiori, en termes de sous-espèce «faible».

Il ne peut être question d'imposer brutalement l'emploi de l'une de ces sous-espèces mais il faut souligner l'incohérance qui résulte de l'emploi de sous-espèces différentes et: a) demander aux auteurs et réviseurs de préciser désormais quelle sous-espèce ils utilisent, en citant si possible les données nécessaires pour «convertir» facilement une sous-espèce en une autre (étude biométrique des caractères distinctifs, nombre d'individus séparables des sous-espèces les plus proches) — b) choisir l'une des sous-espèces.

Je suis personnellement partisan de la sous-espèce de la règle de 100% telle qu'elle est employée par une minorité de lépidoptéristes rhopalocéristes (Warren, Higgins, van Son).

Ce choix peut être justifié ainsi à mon avis:

1) il est désormais évident que le «baptême» de chaque population différente d'une espèce conduit à une «impasse» car nous savons désormais (cf. Mayr, 1942) qu'il n'existe pas deux populations identiques. L'isolement en une catégorie taxonomique de toute population différente conduira donc à la pulvérisation de certaines espèces en minuscules entités peu déterminables. Ainsi les populations désignées par Verity (1950) sous les noms de *protea* Vrty, *protea trans* ad. *subpatycosana* Vrty, *subpatycosana* Vrty *trans.* ad *protea*, *subpatycosana* Vrty et *patycosana* Vrty correspondent certainement a un cline d'Huxley arbitrairement «sectionné».

2) le désir d'éviter l'écueil d'une trop grande pulvérisation a conduit certaines disciplines à adopter des limites conventionnelles (règles de 50 ou de 75%). Cette solution était séduisante au temps de la taxonomie statique (ni trop ni trop peu de sous-espèces) mais elle ne convient pas à mon avis à la taxonomie évolutive car les sous-

espèces ainsi déliminées ne correspondent à aucune étape réélle de l'évolution. A trés juste titre Edwards (1954) écrit avec ironie «the number 75 is the magical panacea for our systematic troubles» et pense que «It will no doubt amuse and amaze future systematists».

3. le défaut le plus grave des sous-espèces «faibles» est cependant à mon avis qu'elles conduisent à isoler dans la même catégorie taxonomique des populations qui correspondent à des étapes très différentes de l'évolution. L'obligation d'employer le trinôme des Règles exige en effet de placer dans ce cas sur un même niveau des populations «sans autonomie morphologique» (telles que *H. charitonius vasquezae* dont aucun individu n'est caractéristique de la population) et des populations ayant atteint cette «autonomie» (telles que *H. ch. peruvianus* dont tous les individus sont caractéristiques de cette population). Or l'existence d'une «différence» entre certains individus d'une population est un phénomène réversible soumis à la fluctuation génique (cf. Teissier, 1952) tandis que l'apparition d'une différence entre tous les individus d'une population est un phénomène irréversible et donc une étape décisive de l'évolution. Warren, le promoteur de la sous-espèce «forte» chez les Rhopaloceres, écrit dans le même sens en 1958 que tant que le «subspecific strain still persists» . . . il peut «increase again at any time».

4. la meilleure méthode consiste donc à mon avis à isoler sous forme de sous-espèces seulement les populations «à autonomie morphologique» de la règle des 100% et à effectuer une étude analytique du polymorphisme (ou plus simplement du morphisme, cf. Huxley, 1955) au sein de ces populations.

5. en tant que lépidoptériste il me semble que l'emploi d'une catégorie infrasubspécifique (la morphe) est une méthode commode pour effectuer cette analyse car les monographies de Warren (1936) et d'Higgins (1941—1955) ont clairement montré que l'absence «d'autonomie morphologique» entre population est souvent provoquée par la présence en proportions différentes de formes passablement tranchées. Il est cependant évident que tout autre procédé (diagrammes, pourcentages, classes, clines . . .) peut être utilisé et convient peut-être mieux pour les groupes d'animaux dépourvus de caractères distinctifs «spectaculaires» pour l'œil humain.

6. il me faut cependant préciser que l'emploi de la règle des 100% manque de souplesse chez Warren et van Son car ces auteurs ne prennent pas en considération les zones d'intergradation secondaires dans l'établissement des coupes subspécifiques. Ainsi contrairement à van Son (1949) je considére que *Mylothris chloris chloris* F. et *Mylothris ch. agathina* Cr. sont des sous-espèces distinctes bien qu'ils se trouvent ensemble, avec des intermédiaires, dans l'Ouganda tandis qu'ils s'excluent sur d'immenses territoires en Afrique. La cohabitation est ici un phénomène secondaire survenant après un isolement antérieur.

Il me reste à exprimer ma respectueuse reconnaissance a Mr G. Teissier, Professeur à la Sorbonne, qui a bien voulu me conseiller pendant le présent travail et en revoir le manuscrit.

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RÉALITÉ CONTRE RÉALISME

S. G. KIRIAKOFF, Gand

On dit souvent que le système biologique moderne est arrivé à une certaine stabilisation et que les changements à venir ne se rapporteront plus qu'à des détails, les grands traits étant définitivement fixés. On dit aussi que cette stabilisation est le résultat des efforts des systématiciens à ranger les organismes suivant leurs affinités naturelles. Le système biologique, tel qu'il est accepté aujourd'hui par les spécialistes, serait donc, du moins en très grande partie, un système naturel.

Une acceptation du système simplement parce qu'on le dit «naturel» n'est pourtant pas une attitude scientifique. Il est donc indispensable de préciser la nature des affinités en question et le sens du mot «naturel».

Qu'est-ce que l'affinité? Le Larousse universel nous dit: «conformité, ressemblance, analogie . . . rapport, liaison». Cette définition n'en est pas une car trop de choses disparates en font partie. En disant affinité naturelle on essaie de pallier cette imprécision, mais en vain car «naturel» veut dire «conforme à l'ordre de la nature», tandis que «ordre» veut dire «règle établie par la nature» . . . Il est vrai que «naturel» veut aussi dire «conforme à la raison», et c'est en définitive ce dernier sens qui prévaut. Les affinités naturelles sont donc, parmi les faits constatés par nos sens, ceux que notre raison choisit parce qu'ils sont l'expression de conformités, de ressemblances ou d'analogies entre les choses observées. Le fait p. ex. d'extrémités transformées en nageoires chez les Poissons et chez certains Insectes aquatiques peut conduire à la supposition qu'il existe des «rapports» ou des «liaisons», donc des «affinités» entre ces divers organismes. Notre raison nous dira qu'il s'agit ici d'analogies, de même qu'elle nous dira qu'il s'agit d'homologies lorsqu'il s'agit d'extrémités de diverses espèces de Poissons. Elle nous dira aussi que ce ne sont que ces dernières ressemblances qui laissent supposer l'existence d'«affinités naturelles».

Ce concept de «ressemblance» englobe donc les concepts de «conformité», d'«analogie», d'«homologie» etc. Mais dans tous les cas, notre raison se substitue à la nature, celle-ci agissant dans notre pensée comme si elle était dotée d'une raison semblable à la nôtre. Dans tous ces cas, c'est notre raison qui juge de la valeur des ressemblances comme indicatrices d'«affinités naturelles». Et le système dit «naturel» que nous établissons à partir de ces affinités, n'est naturel que parce que notre raison le juge tel, sans que nous ayons le moyen de prouver autrement que par raisonnement que le jugement de la nature coïncide avec le nôtre. Ce ne sont en fait que les apparences qui soient en notre faveur.

Les ressemblances qui sont notre seul critère ne relient évidemment que les faits qui peuvent être perçus, constatés et enregistrés par nos sens Ces faits font partie des objets que nous observons, et puisqu'il s'agit ici d'observations biologiques, ces objets sont des organismes, et ces faits sont les caractères propres à ces organismes.

Notre «système naturel» est donc fondé sur les ressemblances entre les caractères des organismes. Quels sont parmi ces caractères ceux à considérer? C'est ici que notre raison doit intervenir et juger s'il s'agit d'homologies, d'analogies ou de simples accidents. Si deux Coléoptères ont perdu par accident une patte postérieure, notre raison s'opposera à leur rapprochement, pour ce motif, dans le système.

Il s'agit dans la pratique presqu'exclusivement de caractères tirés de la structure même de l'organisme. Celle-ci peut être envisagée de manières différentes. Nous distinguons entre les caractères structurels propres, soit externes (exomorphologiques) soit internes (endomorphologiques ou anatomiques); les caractères physiologiques qui ne peuvent exister que parce que l'organisme possède une structure déterminée; les caractères écologiques qui sont généralement considérés comme une conséquence des caractères physiologiques; les caractères biochimiques qui sont aussi fonction d'un aspect de la structure, à savoir la composition chimique de l'organisme; les caractères éthologiques, également coordonnés avec la structure; enfin, les caractères génétiques, structurels eux aussi dans un sens, car la garniture chromosomique est une structure, mais qui conditionnent, d'autre part, le rest de la structure et tous les caractères en résultant.

La somme de ces divers caractères est donc extraordinairement compliquée. C'est ce qu'on appelle la Gestalt ou l'holomorphe (Hennig, 1950). Nous y ajoutons la distribution des organismes dans l'espace sensu lato, l'ensemble des caractères spatiaux étant les caractères chorologiques.

De tous ces caractères, nous ne connaissons en fait généralement que les caractères morphologiques (sensu lato) et les caractères chorologiques. Ces derniers ne jouant par la nature des choses qu'un rôle auxilaire, ce sont les caractères morphologiques qui forment le fondement le plus souvent exclusif du «système naturel».

L'élaboration d'un système fondé en fait non sur la connaissance positive des processus de la nature, mais sur quelques constatations dont la valeur est appréciée par la raison humaine, n'est pas concevable sans l'etablissement, dès le début, de certaines normes servant de commune mesure et permettant la délimitation de groupes «naturels» d'organismes. A ces normes, on donne le nom de «types», plus exactement types morphologiques. Ce concept (qui n'a rien à voir avec le type des philosophes) est à la base même de tout système fondé sur la raison et sur les affinités que celle-ci juge être «naturelles».

Tout système présentant ces caractéristiques est donc un système typologique. Ce qualificatif est généralement réservé à une catégorie déterminée de systèmes, mais cette distinction est le résultat de la confusion de termes et de concepts dont souffre la systématique biologique moderne. La plupart des systématiciens se réclament de la phylogénétique et nient toute attache avec la typologie. Nous estimons néanmoins que tout système biologique qui est fondé sur le raisonnement autant ou plus que sur les faits, et qui conduit à l'établissement de types morphologiques, fait partie de la catégorie des systèmes typologiques. Car l'«ancêtre» des systématiciens qui se disent phylogénéticiens n'est rien d'autre qu'un type morphologique, un «imaginary being» comme dit Danser (1950), une idée, un produit de l'esprit, donc un élément métaphysique.

Il existe cependant aussi un système phylogénétique, fondé également sur le concept de l'ancêtre. Mais il y a une opposition de principe entre ce système et ceux qu'on qualifie de nos jours, à tort, de systèmes phylogénétiques. Cette opposition résulte de

ce que c'est un fait morphologique qui est à la base du système typologique (ou pseudo-phylogénétique), tandis que le système phylogénétique vrai est fondé sur le fait de la succession chronologique.

Il est vrai qu'un système fondé sur la raison peut difficilement nier ou simplement négliger la filiation, car ce qui satisfait le mieux notre «sense of natural connection» (Danser, 1950) est précisément la filiation ... Si un œuf de Drosophile donne sous nos yeux une mutante «wingless», nous négligerons les différences morphologiques pour reconnaître son origine immédiate du couple normale ayant servi à l'expérience. C'est notre sens de relations naturelles qui se trouve satisfait ici, car il reconnaît (peut-être inconsciemment) que c'est la filiation qui est le critère le plus sûr d'une «affinité naturelle».

Pour concilier ces deux conceptions, on se sert de l'axiome «ressemblance morphologique signifie parenté phylétique», axiome qui est à la base de toute la systématique moderne dite «phylogénétique».

En réalité, cet «axiome» n'est qu'une constatation empirique fondée sur l'observation (un chat donne naissance à un chat), et comme telle elle a tout au plus la valeur d'une règle. Les bons esprits l'ont compris. Nous lisons p. ex. chez Remane (1952): „Ähnlichkeit und Verwandtschaft sind in der Biologie nicht gleiche Begriffe". C'est la méthode des analogies et homologies qui doit permettre de pallier cette constatation peu favorable à la typologie. Mais le fond de la question reste inchangé: ce sont les ressemblances morphologiques qui décident de la place d'un organisme dans le système. On peut donc dire que le principe fondamental des typologistes n'est au fond qu'une méthodique. En typologie pure, nous trouvons bien une distinction entre la théorie et la pratique, entre le principe et la méthode, avec les types morphologiques et le classement satisfaisant notre sens d'harmonie. Cette distinction est oblitérée chez les pseudo-phylogénéticiens précisément parce qu'ils refusent de reconnaître que leur type ancestral n'est rien d'autre que le type morphologique idéal d'un Troll ou Danser.

Il en résulte qu'en système typologique sensu lato un nombre infini ou indéfini d'arrangements de même valeur théorique peut être fait: les systèmes biologiques sont nombreux. Le système actuel reflète nos connaissances d'aujourd'hui comme ceux de Lamarck ou de Cuvier reflétaient les connaissances d'alors, interprétées de deux façons différentes. Ce système est en évolution constante, et cela d'autant plus qu'à côté des faits (morphologiques!) il y a leur interprétation. Ici, nous quittons le terrain strictement scientifique: un fait est objectif, une interprétation est subjective, et même la constatation d'un fait objectif contient un élément subjectif (d'interprétation) dont spéculatif. Aussi bien, tout système typologique offre-t-il un mélange de faits, de théories, d'hypothèses, de spéculations plus ou moins plausibles. Ce caractère d'incertitude et de spéculation est suffisamment net pour que nous puissions affirmer que tous les systèmes typologiques contiennent un élément métaphysique. La «phylogénétique» des auteurs se réduit à une «Gestalt» métaphysique: l'ancêtre supposé être tel à cause de sa ressemblance morphologique supposée avec ses descendants supposés.

Face à cet édifice instable, se dresse le vrai système phylogénétique. Il est seul et il ne peut en être autrement, car il est fondé sur la filiation, sur la descendance, sur un fait purement matériel, objectif et ne pouvant être interprété que d'une seule façon. C'est le fait purement physique de la reproduction qui est à la base de ce système. Toutes les questions de ressemblance, avec leurs contradictions et difficultés, ne jouent ici qu'un rôle secondaire, accessoire, et elles font en réalité partie non de la théorie de la systématique phylogénétique, mais de sa méthodique.

Le système phylogénétique est le seul défendable si l'on se place au point de vue de la dialectique, du matérialisme historique, car il est le seul à posséder une uniformité qualitative, sans possibilité de renversement, étant fondé sur un fait qui reste toujours le même qualitativement: matériel et objectif — la reproduction physique.

Il résulte de ce qui vient d'être dit que le système phylogénétique est aussi le seul qui ne contienne aucun élément métaphysique, donc le seul strictement scientifique, sans parler de ce qu'il répond mieux que tout autre à notre sens des relations naturelles.

Ce splendide édifice a cependant un défaut: nous ne sommes pas en mesure de nous en servir. Cette impossibilité est d'ailleurs purement formelle et provient de manque de connaissances tant sur le passé, où ce manque est pratiquement total, que du présent, où c'est précisément le plus important, la filiation des formes, qui ne se prête qu'exceptionnellement à l'observation directe. Le seul système strictement objectif, strictement scientifique est donc irréalisable dans la pratique. C'est la dure réalité.

Tous les autres systèmes offrent, nous l'avons dit, un mèlange de données plus ou moins certaines et d'hypothèses plus ou moins vraisemblables, et ses taxa du même échelon abritent fatalement des groupements de valeur très inégale. Si nous divisons p. ex. les Hyménoptères en 2 sous-ordres Symphyta et Apocrita, tout en faisant remarquer que seuls les derniers semblent constituer une unité monophylétique, nous reconnaissons que notre système n'est pas «naturel» et par conséquent pas scientifique. Le système est bourré de pareilles incongruités.

Aussi bien, faut-il avoir le courage scientifique de déclarer que nos systèmes ne sont que des fabrications artificielles et que leur valeur est purement pratique. Il n'est pas scientifique de discuter gravement sur la valeur respective et la «dérivation» de divers taxa alors qu'on sait au fond que la discussion et ses conclusions ne reposent que sur des hypothèses. Les faits morphologiques sont réels, mais comme nous l'avons dit, leur interprétation sort presque toujours du cadre de la science pure.

La seule attitude vraiment scientifique est donc, croyons-nous, la confession que l'importance attachée à l'arrangement systématique, à la séquence des groupes, à la valeur hiérarchique des taxa etc., est injustifiée. Le système n'a que la valeur d'un schéma servant à l'dentification d'organismes, et il est temps qu'on le reconnaisse. Cela éviterait des discussions parfaitement oiseuses sur la «classification« etc. Quant aux arrangements pratiques qui résulteraient de pareille reconnaissance, les discuter sortirait du cadre de cet exposé. Nous avons seulement voulu montrer qu'il n'existe qu'un seul système biologique réellement scientifique parce qu'objectif, et que l'impossibilité purement formelle de l'utiliser n'augmente en rien la valeur scientifique (qui est sinon nulle, du moins excessivement douteuse) des systèmes actuels dits «phylogénétiques» qui ne le sont à aucun titre.

WESEN, ANWENDUNGSBEREICH UND NOMENKLATUR DES TAXONS SEMISPECIES

Z. LORKOVIĆ

In meinem Kongreß-Referat über die „Abstufung der reproduktiven Isolationsmechanismen in der *Erebia tyndarus*-Gruppe und deren Systematik" habe ich gezeigt, daß zwischen 5 genauer experimentell untersuchten allopatrischen Formen dieser Gruppe, darunter 4 europäischen, auf Grund der Ausprägung (Stärke) zweier reproduktiver Isolationsmechanismen, der Paarungsisolation und der Sterilität bzw. Lebensunfähigkeit der Hybriden, verschiedene Abstufungen der reproduktiven Isolation vorhanden sind, die von rassischer bis zu spezifischer Ausbildung führen. Auf Grund

dessen mußte eine Form (*illyrica* Lrk.) als geographische Rasse, zwei Formen (*cassioides* R. & Hohenw., *nivalis* Lrk. & Les.) als vollständige, den sympatrischen ebenbürtige Arten, eine Form (*tyndarus*) mit merkbar unvollständiger reproduktiver Isolation als nicht ganz vollständige Art, während eine Form (*calcarius* Lrk.) durch ihre mittelmäßig ausgebildeten beiden Isolationsmechanismen (PI = 31 und StI = 62) weder als Rasse noch als Art bezeichnet werden darf, sondern eine Mittelstellung zwischen der Rasse und Art einnimmt. Wenn diese vollkommen allopatrische Form mit einer der anderen Formen in räumlichen Kontakt käme, besonders mit *tyndarus*, würden mehr als $^1/_3$ normal lebensfähige und zum Teil fertile Hybriden entstehen, wodurch es zu einem bedeutenden Genaustausch kommen würde. Eine fünfte Form (*iranica* Gr. Gr.) zeigte gegenüber den europäischen *calcarius*-♂♂ einen PI-Wert = mindestens 50, so daß auch dieser Form eine systematisch zweifelhafte Wertung zukommt.

Außer diesem Fall in der *tyndarus*-Gruppe ist ein weiterer Fall von mittelmäßig ausgebildeter Fortpflanzungsisolation unter den europäischen Tagfaltern experimentell sichergestellt worden: *Pieris napi* L. und dessen Gebirgsform *bryoniae* Ochs., die ebenso weder als Rasse noch als Art bezeichnet werden darf (Lorković, 1961, im Druck). Unter den nordamerikanischen Rhopaloceren sind *Colias philodice* God. und *erytheme* Boisd., die etwa 10% Hybriden in der Natur bilden (Hovanitz, 1949). Neben diesen experimentell sichergestellten Fällen besteht noch eine Reihe weiterer Formen oder Formenpaare, über deren taxonomischen Status Meinungsverschiedenheiten oder Kontroversen zwischen den Forschern oder Kennern bestehen, wie dies auch in der *tyndarus*-Gruppe der Fall war. Alle diese durchwegs allopatrischen oder fast ganz allopatrischen Formen bilden in ihren Kontaktzonen fragliche Hybriden in fraglicher Zahl und erwecken deshalb starken Verdacht auf unvollständig entwickelte reproduktive Isolationsmechanismen und folglich auf eine mehr oder weniger mittlere systematische Lage zwischen Art und Rasse. Es sollen hier z. B. nur *Papilio podalirius* L. und *feisthameli* Dup., *Pyrgus malvae* L. und *malvoides* Edw., *Spialia sertorius* Hffmgg. und *orbifer* Hbn., *Lysandra coridon* Poda und *hispana* H. S. um nicht zuletzt *Zygaena transalpina* und *angelicae* anzuführen, über deren systematische Wertung die Meinungen unbestrittener Spezialisten stark auseinandergehen (Daniel, 1954, 1955; Alberti, 1956, 1958).

Abgesehen jedoch davon, wie zahlreich solche Mitteldinge zwischen Art und Rasse bereits bekannt sind, schon die Tatsache allein, daß in zwei bisher genauer experimentell untersuchten Gruppen auch mindestens zwei solche unbestrittene Grenzfälle auf den Tag erbracht worden sind (für den dritten Fall *coridon-hispana* entwickelt sich die Sache in ähnlicher Richtung) bezeugt, daß sie häufiger sein werden, als gewöhnlich angenommen wird. Es ist aber ebenso unbestritten, daß der Übergang zwischen Rasse und Art bereits bekannter wäre, wenn für diese Mitteldinge wenigstens ein allgemein anerkannter Name oder Bezeichnung und eine klare Definition vorliegen würde. So scheint z. B. nach den Untersuchungen von Remington (1955) *Papilio polyxenes* F. in der Tat nur eine Halbart von *P. machaon* L. zu sein, während sich die spezifische Differenzierung der ebenso allopatrischen *P. glaucus* L. und *P. rutulus* Luc. gleicherweise noch im Gange befinden würde (Brower, 1959). Trotzdem bezeichnen die beiden Autoren diese Formenpaare als Arten. Es ist deswegen höchste Zeit, über diese Sachen in einem solchen Symposium zu diskutieren und nach Möglichkeit einen internationalen Namen für den Grenzfall zwischen Art und Rasse auszuwählen.

Vor vielen Jahren (1942) habe ich schon die Meinung ausgesprochen, daß der beste Ausdruck für den Übergang von der Rasse zur Art, das Wort „Subspecies" wäre. Dieses Wort ist nämlich höchst unpassend für die geographische Rasse, weil sich diese durch das Fehlen der reproduktiven Isolationsmechanismen prinzipiell von der Art unterscheidet; die Rasse ist somit etwas wesentlich anderes als die Art und folglich ist für dieselbe der Ausdruck „. . . species" inadäquat. Dieser Ausdruck würde am besten

gerade für die unvollständige Speziationsstufe passen, indem die letztere noch nicht die Species ist, aber schon schwache Merkmale der Species zeigt, also unter der Stufe der Species, aber dieser ähnlich ist, eben „Subspecies". Die geographische Rasse ist zwar auch unter der Species-Stufe, hat aber mit dieser noch keine Ähnlichkeit, besonders in der Entomologie, indem den geographischen Rassen die Genitalunterschiede meistens fehlen.

Diesen Überlegungen könnte der Vorwurf gemacht werden, daß es nicht für den Namen, sondern für den Inhalt geht und der Name nur ein Symbol ist. Wenn dieses Prinzip für die Art-, Rassen- und Gattungsnamen gilt und gelten muß und deswegen hie und da auch die unsinnigsten Namen geduldet werden müssen, muß es für die Namen der systematischen Kategorien nicht nur entschieden abgelehnt, sondern gar nicht erlaubt werden. Das geht aus folgendem hervor:

Wenn schon der Ausdruck „Subspezies" in die Systematik international eingeführt ist, würde der zweitbeste Ausdruck für den Übergang zwischen Art und Rasse das Wort „Semispecies" sein, da es eben eine halbwegs entwickelte Art bedeutet. Diesen Ausdruck schuf Mayr (1940), um damit „Arten zu bezeichnen, die sich geographisch ersetzen, morphologisch aber zu stark verschieden sind, um Subspeciese genannt zu werden" (p. 267) und etwas weiter: „Sie zeigen durch ihre Verbreitung und allgemeine Ähnlichkeit, daß sie noch vor kurzem geographische Formen einiger anderer Arten waren, aber während ihrer geographischen Isolation solche Merkmale entwickelt haben, daß die Mehrzahl der Autoren es vorziehen würde, dieselben als gute Arten zu nennen" (l. c. p. 260).

Aus diesen Zitaten geht hervor, daß unter der Semispecies Mayrs sowohl echte als auch unvollständige Arten inbegriffen sein können, da von der reproduktiven Isolation nicht gesprochen wird, dieselbe aber aus dem rein morphologischen Bilde allopatrischer Formen nicht so leicht zu entnehmen ist, der Ausdruck Semispecies aber eben auf etwas solches Unvollständiges hindeutet. Aus diesem Grunde verwendete ich im Jahre 1953 zum erstenmal das Wort „Semispecies" für die morphologisch ziemlich verschiedene, aber reproduktiv nur mittelmäßig isolierte Form *calcarius* der *tyndarus*-Gruppe, da ich voraussetzte, daß Mayr eben deswegen den Prefix „semi" gewählt hat. Im gleichen Sinne verstand die Semispecies als Grenzfall zwischen Art und Rasse auch Huxley (1942, p. 407) und führt das Mayrsche Beispiel der Vögel *Colaptes auratus* und *C. afer* an, welche Vögel „in einem 1200—1300 Meilen langen und 300—400 Meilen breiten Gebiete bei der Mehrzahl der Individuen verschiedene Merkmalskombinationen zeigen". Als zweites Beispiel gibt Huxley den bekannten Fall von Raben- (*Corvus corone*) und Nebelkrähe (*C. cornix*) an, zwischen welchen ebenso eine mehrere hundert Kilometer breite Überschneidungszone mit beschränkter Kreuzung vorkommt.

Wohl fühlte Mayr, daß die Anwendung des Wortes „Semispecies" an echten Arten ungeeignet ist, und spricht in dem Buch von 1953 gar nicht mehr von der Semispecies, sondern nur von der Superspecies, während man eine Erklärung der Semispecies nur noch im Glossary findet: „Die Semispeciese sind Arten, von denen sich eine Superspecies zusammensetzt. Die Semispeciese sind eine besondere Sorte der Species, aber keine von der Species verschiedene Kategorie" (l. c. p. 321).

Es wäre somit zu entscheiden, auf welche von den zwei besprochenen Begriffen der Ausdruck „Semispecies" anzuwenden ist, ob auf echte oder unechte (unvollständige) allopatrische Arten? Nach der Bedeutung des Wortes „Semispecies" kann dieses nur für unechte, unvollständige Arten gelten, da es sonst irreführend wäre und auch schon war. Die echten allopatrischen Arten können nicht darunter verstanden werden. Nun handelt es sich glücklicherweise bei den Mayrschen allopatrischen „Arten" nicht um wirklich echte, vollständig reproduktiv isolierte Arten, da die experimentelle Prüfung solcher Fälle stets mehr oder weniger unvollständige reproduktive Isolation zwischen

solchen „Arten“ entdeckt, so daß keine Schwierigkeit besteht, alle solche Formen als Semispeciese zu bezeichnen. So haben Clarke (1953) und Clarke und Sheppard (1953, 1955) vier parapatrische „Arten“ der nordamerikanischen *Papilio machaon*-Gruppe untereinander künstlich gekreuzt und kommen auf Grund der teilweisen Fertilität der Hybriden in den Rückkreuzungen und dem Vergleich in der Natur zum Ergebnis, daß es „vorläufig vernünftiger erscheint, diese Formen, besonders die oben erwähnten *P. machaon* und *polyxenes*, nicht als gute Arten, sondern wenigstens als extreme Subspecies zu betrachten“. Die Kennzeichnung „Semispecies“ verdienen jedenfalls auch alle diejenigen Formen, welche von verschiedenen Autoren als „incipient species“ genannt werden, die also offensichtlich unvollständig isolierte Formen darstellen, trotzdem aber als Arten angeführt werden.

Eine allgemein gültige Regel, nach welcher Semispeciese ohne Experiment in der Natur erkannt werden, läßt sich nicht geben, da dies von den jeweiligen Verhältnissen an den Kontaktstellen abhängt (dominant oder intermediär vererbte Merkmale, verschiedene Häufigkeit beider Formen, geographische und ökologische Isolationsfaktoren der Kontaktzone usw.). Es wird sich aber doch stets um Rassen handeln, wenn die Hybriden merklich häufiger als die reinen Formen vertreten sind, da in solchem Falle sicher keine bedeutendere reproduktive Isolation vorhanden sein kann. Umgekehrt, wenn in der Kontaktpopulation die reinen Formen überwiegen, dann ist es klar, daß eine starke Isolation vorhanden ist und folglich Semispeciese vorliegen. Schwierigkeiten in der Beurteilung, ob Semispeciese oder bereits Arten vorliegen, setzen erst bei hohen Isolationswerten ein, z. B. wenn nur etwa 10% Hybriden vorhanden sind. Entscheidend wird in solchen Fällen sein, in welchem Maße es zum wirklichen Genaustausch noch kommt, denn wenn die Hybriden hohe Sterilität zeigen, dann wird es zu keinem eigentlichen Genaustausch, sondern nur zu einer ganz unbedeutenden Introgression der Merkmale einer Art in den Genbestand der anderen Art kommen können (Anderson, 1949). Das ist der Fall bei *tyndarus* und *cassioides*, weswegen ich diese Formen als Arten betrachte. Bei hoher Fruchtbarkeit der Hybriden wird es doch zu einem richtigen, aber beschränkten Genaustausch kommen, wie z. B. bei *Colias philodice* und *eurytheme* (Hovanitz, 1949), weswegen ich diese zwei Formen als Semispeciese betrachte. Doch fallen eingehendere Betrachtungen über diese Fragen aus dem Rahmen dieses Vortrages und werden an anderer Stelle besprochen.

Abschließend sei noch erwähnt, daß sich bereits eine einfache Schreibweise für die Bezeichnung der Semispecies gut einzuführen beginnt (Kiriakoff, 1948, 1955; Remington, 1951; Lorković, 1952; Petersen, 1955) und zwar die ternäre Nomenklatur mit dem Namen der Species in runden Klammern, z. B. *Erebia* (*tyndarus*) *calcarius* Lrk., *Pieris* (*napi*) *bryoniae* Ochs. Solche Schreibweise kann nicht mit Synonymie verwechselt werden, da das Synonym der Gattung mit großem Buchstaben, das der Art hinter den geltenden Artnamen in Klammern geschrieben wird. Über andere Schreibweisen wie auch Fragen der Nomenklatur und der Definitionen verweise ich auf die sehr überblickliche Arbeit von Bernardi (1956, 1957).

SCHRIFTENVERZEICHNIS

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DISKUSSION

E. R. REICHL: Besteht der Unterschied zwischen Semispecies sensu Lorković und Subspecies im alten Sinn nicht in der Hauptsache darin, daß wir bei ersteren die Isolationsmechanismen bereits (aus experimentellen Arbeiten) kennen, bei letzteren aber noch nicht, obwohl sie wahrscheinlich von genau der gleichen Art sind?

DAS GEOGRAPHISCHE PRINZIP IN DER TAXONOMIE

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Seitdem im Jahre 1900 bzw. 1926 Otto Kleinschmidt in seiner Formenkreislehre auf die Bedeutung der räumlichen Verbreitung für die taxonomische Gliederung der Großarten hingewiesen hat, hat sich dieses „Prinzip der geographischen Rassenkreise" (wie es Rensch genannt hat) auch in der entomologischen Taxonomie immer mehr eingebürgert. Die Klassifikation erfolgt demnach unter Berücksichtigung der Evolution: Die Merkmale werden genetisch gewertet und die Verwandtschaft wird da, wo eine direkte Ableitung an Hand der Paläontologie nicht möglich ist, nicht nur durch die vergleichende Morphologie, sondern auch durch die Untersuchung der gegenseitigen Lage der jetzigen Verbreitungsgebiete der Formen zu erkennen versucht, also mit Hilfe der Zoogeographie. Dies führt zu einer Taxonomie auf phylogenetischer Grundlage, die ich 1952 „Biotaxonomie" genannt habe, und damit zu einem wirklich natürlichen System.

Genus und Subgenus sind zwar nomenklatorisch die höheren Grundpfeiler des Systems, taxonomisch betrachtet aber mehr oder weniger willkürlich begrenzte Zusammenfassungen „verwandter" Arten. Ihr Umfang wird subjektiv bestimmt und wird je nach der Neigung der Autoren — lumpers and splitters — verschieden groß ausfallen. Genus und Subgenus sollten aber in jedem Falle monophyletisch entstanden denkbar sein.

Die phylogenetisch-taxonomische Einheit des Systems ist dagegen der Formenkreis, den man international verständlich besser als *Superspecies* (sps) bezeichnen sollte (Schilder 1952). Eine Superspecies ist eine Gruppe nahe verwandter Formen, also Arten und Rassen, welche sich in relativ jüngster Zeit aus einer gemeinsamen Stammform entwickelt haben. Sie umfaßt also sowohl Formen, die zeitlich nacheinander gelebt haben, als auch Formen, die heute räumlich getrennt nebeneinander leben.

Die Superspecies ist eine taxonomische Einheit, aber keine nomenklatorische Kategorie: Wenn sie mit einem Genus oder Subgenus zusammenfällt, bildet dessen Name natürlich auch den Namen der Superspecies; wenn eine solche nomenklatorische Einheit aber mehrere Superspecies enthält, dann sollten diese gegeneinander begrenzt werden, unter Verwendung des Namens der jeweils geographisch zentralen Art (Typospecies) als Superspecies-Namen, also nicht der ältesten, weil diese vielleicht gar nicht zu der Superspecies gehört.

Die rezenten Superspecies kann man nach ihrem Formenreichtum und der gegenseitigen Lage der Verbreitungsgebiete der zu ihnen gehörigen Arten und Rassen in 3 Gruppen teilen:

1. Nicht differenzierte Superspecies: Die Superspecies besteht nur aus einer einzigen, wegen enger Verbreitung nicht weiter unterteilbaren Art, die morphologisch weitgehend isoliert dasteht; sie ist meist das Relikt eines in früheren Zeiten weiter verbreiteten und reich gegliederten Formenkreises. Für solche isolierte Reliktarten möchte ich den Terminus *Perspecies* (ps) vorschlagen.

2. Sympatrisch physiologisch differenzierte Superspecies: Die Superspecies besteht aus einer Anzahl nahe verwandter Arten, die auf engem Raume zusammen leben, ohne Bastarde zu bilden. Diese von Buddenbrock „Schizotypen" genannten Arten sind ohne räumliche Isolation durch mutative, sofort tiefgehende Abänderung der physiologischen Affinität entstanden. Ich möchte sie *Conspecies* (cs) nennen.

3. Allopatrisch regional differenzierte Superspecies: Die Superspecies umfaßt eine Anzahl verwandter Arten und Rassen, welche aus einer einzigen Stammart entsprungen sind und sich nach erfolgter Isolation in Teilarealen des ursprünglich weiten Verbreitungsgebietes durch Selektion gebildet haben. Die von den einzelnen Arten und Rassen bewohnten Areale schließen sich daher auch heute noch gegenseitig aus, außer wenn (besonders in Randgebieten lebende) Formen sich frühzeitig zu physiologisch selbständigen Arten entwickelt haben, welche nach Aufhebung der räumlichen Isolation nunmehr in den Lebensraum der Nachbararten vordringen, ohne sich mit ihnen kreuzen zu können. Solche meist peripheren, relativ kleinräumigen Arten einer Superspecies möchte ich als *Juxtaspecies* (js) bezeichnen, im Gegensatze zu der meist weiträumig verbreiteten Hauptart jeder Superspecies, die man *Typospecies* (ts) nennen könnte.

Alle diese genannten Species sind Endstadien der Artbildung. Die physiologische Differenzierung ist vollendet, die Kreuzung nahe verwandter Arten ergibt höchstens ausnahmsweise eine einzige Generation von Bastarden, und die einzelnen Individuen sind stets eindeutig bestimmbar, da wenigstens in einzelnen Merkmalen keine Übergänge vorkommen.

Daneben kommen aber gelegentlich Arten vor, bei denen die physiologische Differenzierung noch nicht ganz abgeschlossen ist: die Kreuzbarkeit mit anderen Arten ist zwar noch erschwert, aber die Variationsbreite der Individuen überschneidet sich mit diesen, so daß einzelne intermediäre Stücke nicht eindeutig bestimmbar sind, wohl aber stets ganze Populationen. Solche „werdende" Arten wurden von Birula Prospecies, von Lorkovicz Semispecies genannt, ich möchte aber dem Worte *Quasispecies* (qs) den Vorzug geben. Auch diese Quasispecies sollten noch binär benannt werden, obwohl sie zu den ternär zu benennenden Subspecies überleiten.

Wenn die Artdifferenzierung noch weniger fortgeschritten ist, wenn also die Taxa in benachbarten Räumen zwar im allgemeinen morphologisch verschieden, aber bei ermöglichter Kreuzung noch unbegrenzt fruchtbar sind, dann sprechen wir von Rassen, Unterarten, Subspecies. Aber der Begriff „Rasse" sollte untergeteilt werden: die Bezeichnung *Subspecies* (ss) sollte für diejenigen räumlich ausgedehnten Hauptrassen reserviert bleiben, die morphologisch so weit differenziert sind, daß die Mehrzahl der Individuen eindeutig bestimmt werden kann, auch wenn ihr Fundort unbekannt ist. Sie zerfallen oft in Rassen zweiter Ordnung, Unterrassen, die man als *Infraspecies* (is) klassifizieren sollte, in Anlehnung an die z. B. von G. G. Simpson mit Erfolg eingeführte Terminologie bei Klassen, Ordnungen usw. der Säugetiere. Logischerweise sollten Infraspecies besser quaternär benannt werden, als wie bisher ternär. Zu diesen Infraspecies sind auch drei Spezialfälle zu stellen:

1. *Locinfraspecies* (lis), d. s. Lokalrassen, die auf engstem Raume wenigstens bei der Mehrzahl der Individuen auffällige Merkmale zeigen, wie sie sonst nur bei großräumigen Rassen vorkommen; manche von diesen könnten allerdings auch Modifikationen darstellen, und wären dann nicht benennungswürdig.

2. *Disinfraspecies* (dis), d. s. die Bewohner der Teilareale einer disjunkt verbreiteten Art, deren Gesamtwohnraum also durch weite unbewohnte Zonen unterbrochen ist. Die Populationen beider Teilareale können noch so ähnlich sein, daß bis jetzt keine sicheren morphologischen Rassenmerkmale gefunden werden konnten; da aber bei solcher disjunkter Verbreitung eine beginnende genetische Differenzierung anzunehmen ist, sollten die Bewohner weit getrennter Areale auch nomenklatorisch auseinander gehalten werden.

3. *Subinfraspecies* (sis), d. s. in analoger Weise die Bewohner verschiedener Häufigkeitszentren einer Art, die durch nur spärlich besiedelte Areale getrennt sind, besonders wenn das Gesamtareal einen Umfang hat, innerhalb dessen die Nachbararten in deutlich unterscheidbare Rassen zerfallen.

Alle diese bisher genannten Formen sind benennungswürdig, weil sie entweder bereits physiologisch differenziert sind, oder weil ihre zusammenhängende, gegen die Nachbarform abgrenzbare Verbreitung für eine genetische Sonderung spricht. Sie sind also gemäß den Internationalen Nomenklaturregeln binär, ternär und eventuell quaternär zu benennen.

Nicht benennungswürdig sind dagegen alle Taxa, die in einzelnen Individuen oder selbst Populationen mehr oder weniger zerstreut zwischen der „Normalform" des übergeordneten Taxon vorkommen. Man kann bei diesen wenigstens drei Gruppen unterscheiden:

1. Als *Morphe* (m) bezeichnet Julian Huxley kreuzbare Mutationen, die über das ganze Areal einer Art verbreitet sind und in den einzelnen Populationen in wechselndem Anteil neben einander leben, wobei die extremen Varianten häufiger sind als intermediäre. Dazu gehören auch der Saisondimorphismus usw.

2. Als *Varietas* (v) möchte ich Merkmalskombinationen bezeichnen, die an zerstreuten Orten das Bild der Population bestimmen, an anderen Orten aber fehlen oder nur durch einzelne Individuen repräsentiert sind. Sie können milieubedingte Modifikationen sein, aber auch ökologisch selektionierte Parallel-Mutationen.

3. Als *Aberratio* (a) sollten alle abweichenden Individuen bezeichnet werden, welche sporadisch einzeln auftreten und als nicht erbliche Modifikationen, ja oft als pathologische Erscheinungen zu erkennen sind.

Diese drei letztgenannten Kategorien sollten nicht benannt, sondern nur durch stetig wiederkehrende Abkürzungen oder selbst „Namen" bezeichnet werden, die nicht unter die Internationalen Regeln der Zoologischen Nomenklatur fallen.

Alle hier vorgeschlagenen neuen Termini können durch je 2—3 Buchstaben abgekürzt und in Klammern dem Art- bzw. Rassennamen vorangestellt werden, ohne daß sie die binäre Nomenklatur stören; sie beleuchten aber eindeutig die stammesgeschichtliche Bedeutung der einzelnen Taxa.

Beispiele nach F. A. Schilder, Einführung in die Biotaxonomie, Jena 1952: sps: *Cicindela lunulata* (p. 56); ps: *Apteroessa grossa* (p. - -); cs: *Pogonostoma* (p. 94) und *Derocrania* (p. 61); ts: *Cicindela hybrida* (p. 124); js: *C. lewisi* (p. 124) und *gallica* (p. 92); qs: *C. hispanica* + *turcica* (p. 77); ss: *C. maritima* + *restricta* (p. 125); is: *C. maritima* + *kirgisica* + *finmarkica* (p.p. 125); lis: *C. saphyrina* (p. 84); dis: *C. sexpunctata* + *tripunctata* (p. 76); sis: *C. sylvatica* + *pseudotypica* (p. 60); m: *Adalia bipunctata* (p. 98); v: *Cicindela germanica*, schwarze Form bei Leipzig (p. - -); a: *C. campestris*, blaue Form (p. 84).

Synopsis der vorgeschlagenen Termini der Taxa

	In jüngerer Zeit monophyletisch entstandene Gruppe von Arten und Rassen	Superspecies	sps
1	Verbreitung zusammenhängend (selten großräumig-disjunkt)	*benennungswürdige Taxa*	
2	— physiologisch schon differenziert: nicht kreuzbar, Übergänge fehlen	Species	s
3	— — morphologisch isolierte Relikte	Perspecies	ps
3′	— — sympatrisch, kleinsträumig	Conspecies	cs
3″	— — allopatrisch, weiter verbreitet		
4	— — — zentral lebend, relativ großräumig	Typospecies	ts
4′	— — — peripherisch lebend, relativ kleinräumig	Juxtaspecies	js
2′	— physiologische Differenzierung noch unvollendet	Quasispecies	qs
2″	— physiologisch noch nicht differenziert: unbegrenzt kreuzbar, Übergänge häufig		
5	— — Merkmale deutlich; großräumig	Subspecies (s. str.)	ss
5′	— — Merkmale noch undeutlich, *oder* kleinsträumig	Infraspecies	is
6	— — — Merkmale deutlich, nur an einem Ort lebend	Locinfraspecies	lis
6′	— — — Merkmale oft kaum erkennbar; weiter verbreitet		
7	— — — — in Teilarealen bei disjunkter Verbreitung der Species	Disinfraspecies	dis
7′	— — — — in Häufigkeitszentren bei weitester Verbreitung der Species	Subinfraspecies	sis
1′	Verbreitung der Individuen oder Populationen zersplittert	*nicht benennungswürdige Taxa*	
8	— sympatrisch weit verbreitete Mutationen (Zwischenformen seltener)	Morphe	m
8′	— sporadisch lokal das Populationsbild bestimmend (Zwischenformen häufig)	Varietas	v
8″	— einzelne modifikativ oder pathologisch abweichende Individuen	Aberratio	a

DISCUSSION

G. BERNARDI: La véhémence ou la crainte manifestées par certains auditeurs à propos des exposés de Lorkovic, Schilder et de moi-même ne doit pas surprendre. Cela s'est produit chaque fois que les progrès de la taxonomie ont exigés l'emploi de catégories nouvelles.

Laubmann (1932, *Alauda*, 4: 378) a déjà insisté sur ce phénomène psychologique à propos de la subspecies: «Ce n'est qu'après des luttes prolongées, parfois d'une extraordinaire exaspération — je ne rappellerai que la controverse maintenant a peine compréhensible pour nous, suscitée par la notion de subspecies ... — que la nomenclature ternaire emporta de haute lutte la haute et inexpugnable position que vu sa valeur d'expression des connaissances et faits zoogéographiques personne ne songe plus, désormais, à lui contester».

Malgré la méfiance en face des innovations, l'esprit de routine ou les difficultés d'adaptation je crois donc qu'au moins une partie des catégories taxonomiques modernes s'imposera parsque ces catégories nous permettent, comme la subspecies, de donner une image plus exacte de l'évolution.

Aussi je me permets de regretter qu'aucune tentative n'a été faite aujourd'hui pour unifier la notation de ces catégories. En pratique des auteurs de plus en plus nombreux utilisent de telles catégories mais d'une manière anarchique faute d'un accord international. Il est même désormais impossible de comprendre les notations de ces auteurs sans étude approfondie de l'«historique» de toutes les tentatives de notation. Par suite même les adversaires des catégories taxonomiques modernes ont intêret à voir adopter une notation unique pour chaque catégorie ne serait que pour leur permettre de «traduire» facilement les notations «hérétiques» en notations classiques. Pour les partisans de la taxonomie évolutive l'enjeu de l'unité est évidemment plus important: il s'agit de transformer les catégories taxonomiques en un instrument de travail pour l'étude de l'évolution au d'être un obstacle à cette étude.

BRAUCHEN WIR INTERNATIONALE EMPFEHLUNGEN ZUR HANDHABUNG DER KLASSIFIKATION IN DER ZOOLOGISCHEN SYSTEMATIK?

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Die Frage soll untersucht werden im Zusammenhang mit dem Problem der Kleingattung, das seinerseits wieder in enger Beziehung steht zum Zweck des binominalen Benennungsprinzips der Spezies und zur Stabilität der Nomenklatur.

Aus der Vielzahl kritischer Stimmen seit mehr als 100 Jahren sei nur zitiert, was R. Richter 1948 im Kommentar der Nomenklaturregeln schreibt:

„Die Zerfaserung, die in manchen Abteilungen des Tierreiches fast jede Art zu einer selbständigen Gattung machen möchte, bringt der Wissenschaft nur Nachteile. Man entwertet dadurch den Gattungsnamen zu einer bloßen Vorsilbe des Artnamens, gibt die Errungenschaften von Linnaeus binaerer Nomenklatur unbewußt wieder auf und sinkt sogar noch unter den Stand von 1758 zurück."

Andererseits schreibt der gleiche Autor zu Artikel 7 der Regeln:

„Die Wissenschaft, nämlich die Taxonomie, kann zwar vor einer unbegründeten Aufspaltung der Gattungen warnen und tut dies mit zunehmendem Nachdruck, durch äußerliche Vorschriften nomenklatorischer Art ist aber eine Reglementierung wissenschaftlicher Taktfragen nicht möglich."

In kurzen Worten ergibt sich hieraus der heute noch allenthalben vertretene Standpunkt:

Die klassifikatorische Einordnung der Verwandtschaftsgruppen von Arten in das hierarchische System ist Wissenschaft, und in ihrem Bereich gilt grundsätzlich die wissenschaftliche Meinungsfreiheit, die weder durch Reglementierung noch durch Empfehlungen mit ihrer suggestiven Wirkung beeinflußt werden darf.

Diese These ist nunmehr auf ihre Richtigkeit zu prüfen.

Es wird heute fast allgemein und mit Recht anerkannt, daß die höheren Kategorie-Begriffe des Systems, wie Untergattung, Gattung, Klasse usw., keine natürlichen,

sondern nur menschliche Ordnungsbegriffe sind. Eine gewisse Denkschwierigkeit ergibt sich dabei aber aus dem Umstand, daß die Verwandtschaftsgruppen, die wir diesen Ordnungsbegriffen zuteilen, zweifellos natürlichen Charakter haben. Eine Lösung dieses Widerspruchs finden wir rasch, wenn wir streng den Allgemeinen und den Speziellen Begriff als Denkprinzipien unterscheiden. Es ergibt sich dann der Satz: „Die natürlichen Verwandtschaftsgruppen von Arten sind die speziellen Funktionen der einzelnen Kategorienstufen, die ihrerseits allgemeine menschliche Ordnungsbegriffe sind."

Aber auch dieser Satz gilt offenbar nur mit Einschränkung, denn die Kategorienstufen sind wieder Funktionen der Stammbaumverzweigungen in vertikaler Schau und insoweit zweifellos natürlich.

Hier muß jetzt die Unterscheidung zwischen einem natürlichen (idealen) und vielen künstlichen Stammbäumen einsetzen. Dann gilt der weitere Satz: „Nur ein künstlicher Stammbaum entspricht dem jeweils gehandhabten Kategoriensystem der Klassifikation.

Zwischen System, künstlichem und natürlichem Stammbaum gilt ferner die Beziehung: Ausgehend von den wenigen nomenklaturrechtlich obligatorischen Kategorienstufen des Systems bewirkt eine zunehmende Verfeinerung der Unterabstufung auch eine zunehmende Annäherung an die Verhältnisse des natürlichen Stammbaums. Aber diese Annäherung kann auch in der Theorie nie zur vollen Deckung führen, weil in der Natur nicht das hierarchische Teilungsprinzip menschlicher Ordnung gilt, also der sprunghafte Ersatz einer Art durch mehrere neue Folgearten, sondern das Abstammungsprinzip, also das primäre Nebeneinander von Mutterart und Tochterarten, herrscht. Dies ist eine einfache Folge der unterschiedlichen Evolutionsgeschwindigkeit schon bei der Artumbildung, noch viel deutlicher dann bei den höheren Verwandtschaftsgruppen des Systems. Auch hier ist Teilung in gleichwertige Zweige nur Grenzfall.

Der Hauptunterschied zwischen System nebst künstlichem Stammbaum und dem natürlichen Stammbaum ergibt sich somit ebenfalls aus der unterschiedlichen Evolutionsgeschwindigkeit. Hierbei müssen wir wieder streng unterscheiden zwischen Längenwachstum, also Artstufenvermehrung und -zahl und Dickenwachstum, also Stammlinienvermehrung und -zahl.

Grundsätzlich besteht zwischen Artstufenzahl und Kategorienstufenzahl kein Unterschied. Aber in der Praxis des Kategoriensystems unterdrücken wir unzählbare Stufen, teils, weil wir sie nicht erkennen, teils, weil wir zu Ordnungszwecken des Systems ihre Vielzahl als störend empfinden. Hier wird dann schon gewonnene Naturerkenntnis dem Ordnungsprinzip des Systems zuliebe wieder geopfert, woraus bereits klar hervorgeht, daß die Klassifikation keine Naturerkenntnis bedeutet, also keine Wissenschaft ist, im Gegensatz zu der vorangehenden Erforschung der Verwandtschaftsgruppen.

Noch klarer wird der Sachverhalt, wenn wir die horizontale Evolutionsgeschwindigkeit, also Vermehrung der Stammlinien, in die Betrachtung einbeziehen. Aufgabe hierbei wäre es, eine natürliche Koordinierbarkeit aller Stufenabschnitte bei allen Stammlinien durch das ganze System hindurch zu erreichen. Die unterschiedliche Evolutionsgeschwindigkeit versagt uns dies vollständig.

Wir wollen die Sachlage jetzt kurz an einzelnen Fällen prüfen und unterscheiden dabei den Fall des gegebenen Zustandes im System und die Fälle von Veränderungen in ihm.

Bei einem Entwicklungszweig von Arten als natürlicher Verwandtschaftsgruppe sollen 6 Abstufungen Spezies, Subgenus, Genus, Tribus, Subfamilie und Familie unterscheidbar sein. Auf der Familienstufe soll, gekennzeichnet durch Basishomologien, ein Nachbarzweig mit nur drei erkennbaren Abstufungen entspringen. Von ihnen

liegen Familien- und Speziesstufe, d. h. Anfang und Ende fest, nur eine Stufe ist also frei, die wir wahlweise mit Subgenus, Genus, Tribus oder Subfamilie des Nachbarzweiges koordinieren können. Keine dieser Stufen hat ein natürliches Vorrecht, da sie keine Naturgegebenheiten mit speziellen Merkmalen sind. So wählen wir stets nach menschlichen Ordnungsprinzipien, und diese Wahl fällt zwangsläufig auf die Gattungsstufe, weil sie die einzige nomenklaturrechtlich bevorzugte Stufe zwischen Spezies und Familie ist.

Dies führt sofort zu der sehr bemerkenswerten Feststellung, daß die Nomenklaturregeln ständig den sogenannten wissenschaftlichen Takt reglementieren, also die Klassifikation beeinflussen, obwohl sich ein solcher Einfluß gegen das Grundprinzip in der Aufgabe der Internationalen Nomenklaturkommission richtet.

Aber die Situation kann noch krasser liegen.

Gelegentlich ist folgender Fall realisiert:

Tribus-, Genus-, Subgenus- und Artenkreisstufe eines Zweiges sind besetzt. Die Artenkreisstufe sei durch nächstverwandte vikariante Arten gekennzeichnet. In einem relativ isolierten und daher schon auf Tribusstufe entspringenden Nachbarzweig sind nur zwei vikariante Spezies ausgebildet. Sie sollten somit nach wissenschaftlicher Einsicht dem Artenkreis des Nachbarzweiges koordiniert werden. Aber wir stufen sie bedenkenlos als Genus ein, w e i l w i r n u r d i e s e m e i n e n E i g e n n a m e n g e b e n. Hier richten sich also Klassifikation und Nomenklatur geradezu gegen die wissenschaftliche Erkenntnis.

Die einfache Klärung dieses Widerspruchs ist dadurch möglich, daß wir Klassifikation wie Nomenklatur als menschliche Ordnungsbegriffe erkennen und hier dann den Kennzeichnungsbelangen der Nomenklaturregeln den Vorrang geben vor solchen der Klassifikationsordnung. Nur im Stammbaumschema könnte die zutreffende Koordination richtig zum Ausdruck kommen, allerdings mit dem Vorbehalt der unbekannten Größe „Evolutionsgeschwindigkeit“.

Betrachten wir jetzt noch die Situation bei Veränderungen unserer taxonomischen Kenntnisse. Hier bestehen drei Hauptmöglichkeiten:

a) es erfolgt nur andere Merkmalsbeurteilung,
b) es tritt erweiterte Merkmalskenntnis ein,
c) es kommt zur Vermehrung der Artenzahl.

Im Fall a) wollen wir Subgenus- zu Genusmerkmalen umwerten. Ist dann die Genusstufe schon besetzt, so wird ihr Inhalt in eine höhere Stufe abgedrängt und so fort. Es tritt eine Verschiebungslawine ein, die in der Regel nur dadurch gebremst wird, daß der bisherige Inhalt einer Stufe auf eine unbesetzte, in der Regel namenlose Stufe kommt. Wissenschaftliche Erkenntnis ist mit alledem nicht gewonnen, nicht einmal Ordnung und Übersicht, diese sind vielmehr empfindlich gestört, denn die benannte Stufe Untergattung wird frei und eine nicht gekennzeichnete Stufe wird besetzt.

Nur Merkmalsumwertung auf der Basis von Homologie und Konvergenz, etwa die veränderte Wertung von Basishomologien, könnte eine solche Verschiebung rechtfertigen, weil dann veränderte Subordinationsverhältnisse an der Wurzel von Verzweigungen eintreten. Sie sind aber in seltensten Fällen der Grund von Veränderungen im System, die wir vornehmen.

Betrachten wir jetzt Fall b).

Erweiterte Merkmalskenntnis ist Ausdruck echten wissenschaftlichen Fortschritts, der aber als verfeinerte Unterabstufungen und Verzweigungen, also Vermehrung von Verwandtschaftsgruppen und Kategorienstufen, unbegrenzt nur im Stammbaum-

schema zum Ausdruck kommt. Erinnert sei an die bedeutende Verfeinerung der Abstufungen, die oft durch vergleichende Untersuchung der Genitalmerkmale von Insekten erreicht wird.

In der Klassifikation wird diese Erkenntnis leider oft durch Verschiebung der Kategorienstufeninhalte ausgedrückt, wobei dann wieder die Gattungsstufe aus nomenklaturrechtlichen Gründen besonders betroffen wird. Hier haben wir die Hauptwurzel des Übels der Kleingattung.

Betrachten wir schließlich noch Fall c), den Artenzuwachs. Er bedeutet zugleich auch Merkmalszuwachs mit allen schon erörterten Konsequenzen. Als wesentlich neu kommt nur hinzu die Vergrößerung der Gruppeninhalte an Arten. Daraus ergeben sich zunächst wissenschaftliche Konsequenzen, wie uns am eindringlichsten die ungeheure Vermehrung bekannter Arten mit der Folge entsprechender Vergrößerung und Vermehrung von Verwandtschaftsgruppen aller Stufen seit Linnaeus zeigt. Als Konsequenz folgt ferner, genau wie bei der Vermehrung unserer Merkmalserkenntnis, daß auch die Stufen selbst vermehrt werden können, da ja auch mehr Stammbaumunterverzweigungen eintreten. Im Kategoriensystem hat andererseits nur solche Vermehrung Zweck, die auch international gültig mit Kennzeichnung der neuen Stufen verbunden ist. Aber dies ist historisch gesehen nicht im richtigen Verhältnis zur gewachsenen Artenzahl geschehen, besonders nicht bei den nomenklaturrechtlich obligatorischen Stufen. Der Grund liegt einfach darin, daß die allgemeine Ausdrucksmöglichkeit mit den Mitteln des Systems eine solche Vermehrung schlecht erlaubt und auch die Systemübersicht beeinträchtigt. Damit sind wir aber bereits wieder mitten in den Problemen menschlicher Zweckprinzipien, wie sie die einleitend zitierten Ausführungen von Richter zum Problem der Kleingattung klar hervorhoben.

Wie wenig das System Naturerkenntnis bedeutet, wie stark es vielmehr diese gelegentlich geradezu verzerrt, mag schließlich noch folgendes Beispiel zeigen.

Wir erwähnten schon, daß die Ungenauigkeit der Übertragung des Kategoriensystems auf den idealen Stammbaum bereits bei der Speziesstufe beginnt. Die fast immer geltende Unkenntnis der genauen Abstammungsbeziehungen läßt uns über diesen kleinen Fehler leicht hinwegsehen. Aber in höheren Gruppenbereichen des Systems ist die Lage, wie ebenfalls schon bemerkt, gelegentlich viel klarer. Von den Vögeln wissen wir genau, daß sie nur ein Tochterzweig der Reptilien sind. Trotzdem stufen wir beide entgegen der Stammbaumerkenntnis als Klassen im System gleich. Ursachen sind reine Ordnungsprinzipien, nämlich herkömmlicher Gebrauch sowie bequeme Merkmalswertung und dementsprechend -übersicht, dazu vielleicht auch der Gesichtspunkt, daß das System ja sowieso die Wissenschaft zu Konzessionen nötigt, wobei es dann im Ausnahmefall selbst auf größere Konzessionen auch nicht ankommt.

Diesen Ausnahmefall im Extrem haben wir dann da, wo Forscher der Meinung sind, der Mensch mit seinen aufdringlichen Sondermerkmalen solle nicht mehr wie bisher als Gattung des Tierreiches eingestuft werden, sondern als Menschenreich dem gesamten Tierreich koordiniert sein. Hier haben wir zugleich die äußerste Diskrepanz zwischen Stammbaumerkenntnis der Naturwissenschaft und menschlichen Ordnungsprinzipien der Klassifikation.

Die Konsequenz aus alledem im Sinne unserer eingangs gestellten Frage ist sehr klar. Nicht nur ein natürliches Ordnungsprinzip, sondern vor allem rein menschliche Zweckprinzipien bestimmen die Klassifikation. Sie kann also insoweit auch keine wissenschaftliche Meinungsfreiheit beanspruchen. Man verwechselt diese vielmehr mit praktischer Meinungsfreiheit, die aber nicht zulässig ist, solange die menschliche Ordnung irgendwelcher Dinge an einen vereinbarten Zweck gebunden wird.

Aber welches soll dieser Zweck in unserem Falle sein?

Mit guten Gründen könnte man in der Geologie für feinstratigraphische Zwecke und unterstützt durch den mangelhaften Merkmalseinblick bei Fossilien der Kleingattung vor dem Prinzip der Großgattung den Vorzug einräumen, wobei dann der reine Übersichtszweck des Systems nachgeordnet bliebe.

In der Rezentsystematik fällt der stratigraphische Zweck aus und es verbleiben nur zwei Prinzipien, die aber einander weitgehend widersprechen:

a) Kennzeichnung durch Gattungsnamen für kleinste Verwandtschaftsgruppen als Ausdruck der Spezialistenarbeit, und
b) Kennzeichnung durch Gattungsnamen für größere oder herkömmlich umgrenzte Gattungsinhalte als Ausdruck bestmöglicher Übersicht und weitgehend stabiler Ordnung des Systems.

Mit v. Wettstein und anderen Forschern wird behauptet, daß durch die Kleingattung Verwandtschaft nicht ausgedrückt, sondern unkenntlich gemacht wird, weil der Gattungsname dabei mehr und mehr zur Vorsilbe des Artnamens herabsinkt (Richter l. c.).

Nomenklatur wie Klassifikation sind nicht Naturwissenschaft, sondern nur ihre Diener zum Zweck der Verständigung und Übersicht. Im Zuständigkeitsbereich der Internationalen Nomenklaturkommission sind das Ergebnis dieser Einsicht die Nomenklaturregeln. In der speziellen Klassifikation kann es, ihrem Wesen entsprechend keine Reglementierung ähnlicher Art geben. Wohl aber sind Richtlinien oder Empfehlungen der allgemeinen Handhabung notwendig, und in Sonderheit erhebt sich die Forderung, durch internationale Vereinbarungen den Leitzweck des Systems festzulegen, an dem sich dann die Taxinomen für die allgemeinen Gesichtspunkte ihrer speziellen Arbeit orientieren können. Erst dann wird auch die Nomenklatur zur bestmöglichen Stabilität gelangen.

DISKUSSION

MAYER, Berlin-Dahlem: In der Angewandten Entomologie hat man jetzt vielfach begonnen, die Verständigungsschwierigkeiten durch Anwendung deutscher Vulgärnamen für Schadinsekten zu überwinden.

ROBERT R. SOKAL, Lawrence, U.S.A.: Es ist doch erstaunlich, wenn erst im Jahre 1960 alle diese Grundfragen ernsthaft zur Diskussion kommen.

KLASSIFIKATION UND ERFORSCHUNGSGESCHICHTE

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Wenn man die gegenwärtige Klassifikation des Tierreiches vergleichend betrachtet, dann ergeben sich erhebliche Ungleichwertigkeiten in der Fassung der Kategorien; dies betrifft vor allem die generische Gliederung. Verschiedenartige Gründe bedingen diese Verhältnisse. Von diesen wollen wir hier den Faktor „Erforschungs-Geschichte" näher untersuchen und dabei vor allem die Situation noch immer mangelhaft bekannter Gruppen besprechen. Zur Diskussion der gegenwärtigen Verhältnisse ist zunächst eine historische Betrachtung notwendig.

Hierbei wollen wir die Erforschungsgeschichte des Tierreiches artificiell mit dem Jahre 1758 beginnen lassen, dem Erscheinungs-Datum von Linnaeus' Systema naturae

(x. ed.). Bereits in diesem Werk, mit dem die internationale Zoologische Nomenklatur einsetzt, stoßen wir auf ganz erhebliche Ungleichwertigkeiten in der Handhabung der Kategorie der Gattung. So ist es wohl vor allem dem unterschiedlichen Stand der damaligen Kenntnis zuzuschreiben, wenn Linnaeus z. B. die Coleopteren im heutigen Sinne auf insgesamt 22 Genera verteilte, während er dagegen sämtliche ihm bekannten Spinnen der einzigen Gattung *Aranea* einfügte!

Diese Verhältnisse, denen wir bereits bei Linnaeus begegnen, haben sich bis zum heutigen Tag im Prinzip nicht geändert. Je nach dem Ausmaß der Zunahme der Formenkenntnis der einzelnen Gruppen hat die Klassifikation im Laufe der Zeit eine Verfeinerung erfahren. Gleichsinnig mit praktischen Erfordernissen haben diese wissenschaftlichen Gründe die Einführung neuer und die Restriktion schon bestehender Kategorien verlangt. Diese Restriktionen haben die Umgrenzung ursprünglicher Groß-Gattungen enger gefaßt und näher präzisiert.

Die geschilderten Vorgänge sind aus begreiflichen Gründen bei den verschiedenen Gruppen des Tierreiches verschieden weit fortgeschritten. Während aber Linnaeus und die alten Autoren vielfach am Anfang unserer Kenntnis überhaupt standen, können die neueren Autoren sich in zunehmendem Ausmaß wenigstens auf den Modellfall einzelner, relativ gut durchgearbeiteter Regionen beziehen.

Wir erläutern dies mit einem Beispiel: Linnaeus (1758) kannte nur 8 Diplopoden-Arten, davon 3 europäische. Attems schätzte die Zahl der bis 1926 beschriebenen Arten auf etwa 6300, von denen 1200 auf den europäischen Teil der Paläarktis entfielen. Dies bedeutet, daß der Artenzahl des europäischen Teiles der paläarktischen Region damals für die übrigen Gebiete der Erde eine Artenzahl gegenüberstand, die nur etwa viermal so groß war; und dies bei der gewaltigen Formenfülle der Tropen! Diese Zahlen zeigen, daß wir bei den Diplopoden wie auch in zahlreichen anderen Gruppen noch immer ganz am Anfang der Kenntnis stehen, aber nicht mehr, wie die alten Autoren, am Anfang in jeglicher Hinsicht. Somit bestehen heute bei Fragen der Klassifikation prinzipiell zwei verschiedene Möglichkeiten:

a) In manchen Fällen wird der Bearbeiter beim Studium bisher mangelhaft bekannter Faunen oder Regionen vor allem hinsichtlich der Genera gezwungen sein, mit zunächst weiträumig gefaßten Kategorien zu arbeiten und abzuwarten, bis mit fortschreitender Kenntnis die Zusammenhänge deutlicher werden und so eine straffere Gliederung der ursprünglichen „Sammelgruppen“ erlauben. Diese werden hierdurch der Klassifikation der Faunen-Elemente gutbekannter Regionen zunehmend gleichwertig. Das geschilderte Vorgehen würde dem allgemeinen Ablauf der historischen Entwicklung entsprechen, die bereits erörtert worden ist. Auch die Anwendung der Kategorie „Untergattung“ ist in solchen Fällen oft nicht möglich, man wird mit einem Nebeneinander der Genera auskommen müssen. Die Wertung einer Artengruppe als Subgenus innerhalb eines Genus ist eine viel zu schwerwiegende Aussage, die, gestützt auf eine ausgedehnte Kenntnis der Arten, einen echten Einblick in die Zusammenhänge voraussetzt. Es ist ein weitverbreiteter Irrtum, anzunehmen, die Wertung einer Artengruppe „nur“ als Subgenus sei eine vorsichtigere Aussage, solange nicht sicher sei, daß es sich wirklich um eine Gattung handle.

b) Andererseits kann aber auch der Bearbeiter, ausgehend von der Kenntnis der Faunen-Elemente genauer bekannter Gebiete, ein allgemeines Urteil über die Wertigkeit von Merkmalen gewinnen und dieses dann auf die Klassifikation der ganzen Gruppe projizieren. Ein derartiges Verfahren führt beim Betreten wissenschaftlichen Neulandes aus Gründen der Konsequenz zunächst zu einer größeren Zahl vor allem monotypischer Genera, deren Artenzahl sich erst nach und nach mit fortschreitender Formenkenntnis aufzufüllen beginnt. Ein derartiges Vorgehen, das die zuvor geschilderte allgemeine historische Entwicklung zu überspringen und abzukürzen sucht, wird im allgemeinen nur Spezialisten mit weltweitem Überblick möglich sein.

Es muß der Verantwortung des Bearbeiters überlassen bleiben, welchen dieser Wege er einschlagen kann; meist wird er einen Kompromiß suchen müssen. Bei den großen Schwierigkeiten der Beurteilung bleibt er dabei stets der Gefahr des Irrtums ausgesetzt und muß Fehler machen. Nur durch Erfahrung und Kenntnis, Verantwortungsbewußtsein und straffe Geisteshaltung, also durch subjektiv-menschliche Qualitäten kann dem entgegengewirkt werden.

Ich habe versucht, mit diesen Ausführungen die Situation vor allem in den noch immer höchst mangelhaft bekannten Gruppen des Tierreiches anzudeuten und auf den allgemein unterschiedlichen Stand unserer Kenntnis hinzuweisen. Die Probleme sind infolgedessen von Fall zu Fall verschieden. Unter solchen Umständen scheint es ausgeschlossen, etwa allgemeine Regeln für die Handhabung der Kategorien, insbesondere der generischen Einheiten, überhaupt zu formulieren; hier kann nicht durch eine Art „Patent-Rezept" geholfen und die individuelle wissenschaftliche Entscheidung abgenommen werden.

Die taxionomische Wissenschaft, ein wesentlicher Zweig der Zoologie, drückt ihre morphologischen Erkenntnisse und ihre phyletische Ansicht durch die Klassifikation der Lebewesen aus. Die Nomenklatur dient dabei nur als technisches Hilfsmittel zur Verständigung. Wir wissen, aus zweierlei Gründen kann diese Nomenklatur nicht beständig sein: Verschiebungen aus technischen Gründen kommen immer wieder vor, erzwungen von der Notwendigkeit zur Eindeutigkeit und weltweiter Einheitlichkeit. Daneben bewirken aber auch unterschiedliche taxionomische und somit wissenschaftliche Auffassungen Verschiebungen in der Klassifikation und damit entsprechende Änderungen in der Nomenklatur. Wenn darüber diskutiert wird, ob man diese „Namensänderungen aus wissenschaftlichen Gründen" nicht erheblich einschränken, wenn nicht gar unterbinden könne, so würde dies bedeuten, daß man die Situation eines Hilfsmittels der Wissenschaft (= Nomenklatur) festlegte, indem man die Wissenschaft selbst durch Reglementierung ihrer Freiheit beraubte — durch Einfrieren gegenwärtig für richtig erachteter Situationen eines Entwicklungsprozesses. Selbst wenn die zuvor geschilderte Heterogenität der Verhältnisse wie auch die Situation der Nomenklatur als bloßes Hilfsmittel nicht eo ipso ein derartiges Vorgehen ausschließen würden, so sollte dennoch, aus Achtung vor der wissenschaftlichen Meinung des anderen, niemand in dieser Hinsicht Pläne äußern, die einem Totalitätsanspruch gleichkommen.

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GENERA AND SUBGENERA IN CLASSIFICATION AND NOMENCLATURE

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The recent literature on zoological classification and nomenclature is so rich, that it may seem doubtful if some basic questions on these matters are still in need of being dealt with. As ichthyologist, I am considering them from the point of view of a student of modern fishes; from my own experience in this field, some opinions arise that my

colleagues specialized otherwise will perhaps share only in part or not at all. The important change in the taxonomic concepts that developed in modern times attracted the attention of biologists on the problems of species, subspecies, populations, etc. However, it is more and more important to consider carefully also the higher categories.

Let us deal with the genus. Surely, to day all zoologists share Linnaeus' opinion, admitting that this taxon includes one or more species having the greatest possible number of common characters. We currently speak of genera and generic differences, but—as it is well known—they cannot be defined by objective criteria: the genus is something artificial and leaves much room for personal interpretations. In all the zoological groups many old genera have been more and more split and some modern students have gone so far that a great number of monotypic genera have been established on the basis of trivial morphological characters.

There are "genera" of Percoid fishes proposed just because the "type species" shows small differences in the numbers of the fin rays, the development of fin lobes, the size of the scales or the teeth, etc. Such a procedure makes us wonder what is the value of the genus and where lies the difference between generic and specific characters.

A purely morphological concept of the genus is probably responsible for a so regrettable abuse. If this taxon has to keep a meaning, it must be or aim to be a biological unit also: ethological and ecological features are important as the morphological ones. Let us quote a few significant cases from the Mediterranean fishes. When we say that *Germo* cannot be separated from *Thunnus*, *Smaris* from *Maena*, *Coricus* from *Crenilabrus*, we point out that in such pairs of "genera" recognized by the former ichthyologists there is a basic similarity of structure and way of life. Similar cases are easily found in other groups of animals, as the ducks (*Mareca*, *Dafila* and *Querquedula* are simply *Anas*) and the deers (*Sika*, *Panolia*, *Rusa* are really *Cervus*). On the other hand, there are excellent exemples of genera, the validity of which is well supported also on biological grounds. *Salmo* and *Oncorhynchus* among Salmonidae, *Clupea* and *Alosa* among Clupeidae show, beside morphological features, a different behavior concerning the migratory habits; a geographical separation, however not complete, is to be added in the former case.

If we could extend this sampling and pay a more careful attention to genera or groups of genera, it would appear that the evidence of the "generic characters" of different kinds is not all correlated: in some cases the morphological ones are outstanding, in others they are rather poor but the biological ones are very remarkable.

If we give it a wider interpretation, the genus looks less artificial and express in a better way an objective phenomenon, that is the existence of groups of related species. I avoid stating that "lumpers" or "splitters" are to be preferred: both procedures may be right and both may be wrong. For the same reason, we cannot prefer "large" or "small" genera. It may only be stated that the smallest the genus, the wider must be the gap that separes it from the near ones. What really matters is to define genera as groups of species morphologically and biologically related, having gone through a long process of adaptation to a peculiar environment and way of life. So, the genus gets an evolutionary meaning, being correlated with the phylogeny of the included species.

It has been stated rightly that systematists must be increasingly interested in the behavior of the species, as they will find very useful criteria for taxonomic grouping. This has already been proved by observations on birds and insects, in which some relationship problems have been solved with the help of ethology; generally speaking, however, the behavior has been very little considered in connection with the definition of genera. Of course, it is not easy to follow consistently a procedure rigidly adherent to the views here referred to, as in many cases a student will be compelled to describe

a new genus, at least provisionally, on a merely morphological basis: it is just what still happens for the species, notwithstanding so many talks on the "new systematics". Anyway, a concept of the genus as a morpho-biological unit must be kept in mind when critical or revisional work is done. This taxon will then attract the interest of the general biologists, who now chiefly consider the lower systematic units. Among the many problems concerning the species, that of their relationship is outstanding and here the genus comes necessarily into discussion.

Turning to a related question, we may state that if subgenera are admitted—and they are indeed—they are to be defined according to the same basic concepts. Subgenus is a group of species which show a closer affinity, proved by morphological and biological characters. Lets us remind that among the latter, the geographical distribution is important. Too often the subgenus appears as something of very uncertain meaning and value, just as a compromise between recognizing or not a new taxon of generic rank. Here are very frequent cases in which the artificiality of the classificatory system is well apparent. On the other hand, it does not seem possible to establish rules for distinguishing genera and subgenera: this is a matter left to the judgment of the specialists. I will simply say that if we go on with the definitions of subgenera based on very trivial features of some species, we may wonder if such a taxonomic category really deserves to be kept.

Our zoological nomenclature must express the result of the classificatory work and it is quite sure that a better situation in both nomenclature and classification arise when: 1) no names are created for units unacceptable on classificatory grounds; 2) no changes of well known names are too freely effected for purely nomenclatorial reasons.

DISCUSSION

KEY, K. H. L., Canberra: It is usual to contrast taxonomy and nomenclature as distinct fields. It is true, of course that whether a family name should be changed when the name of its type genus is changed is a purely nomenclatural question, while whether two forms are specifically distinct is a purely taxonomic one. But taxonomy and nomenclature are necessarily so interlinked that we are usually concerned with problems having both a nomenclatural and a taxonomic aspect.

The prime function of nomenclature is to provide a stable and universal hierarchical system of names to correspond with the hierarchical classification of organisms. The goal of stability has been rightly emphasised in recent years, but it must be recognised that stability is in large measure incompatible with advance in taxonomic knowledge. We can, of course, take steps to minimise instability of purely nomenclatural origin. We can agree that the original spelling of a name must prevail under all circumstances and that linguistic considerations must never be allowed to lead to name-changing. It is surprising, though, how many zoologists who profess to be in favour of stability are unwilling to tolerate obvious errors of linguistics or transliteration in names with which they have to deal. There are also very difficult decisions to be made, even within the purely nomenclatural field. For example, which is the less objectionable—to change the name of a well known family, or to have a family name based upon a rejected generic name?

It may also be possible to preserve stability when it is threatened from the taxonomic side. Thus if a well known name is shown as a result of taxonomic investigation to be a synonym of an earlier name that has not been in use for many years, the later name can be protected by suppression of the earlier one. But nothing can be done to prevent name-changing when two well known names are shown to be synonyms, when a species is transferred to a different genus, ot when such a transfer brings about a state of homonymy. As we are able progressively

to eliminate name-changing arising from purely nomenclatural causes and as we gain in our understanding of taxonomic relations, so most name-changing will come to consist of the unavoidable type.

The question then arises as to the means to be adopted for avoiding that part of nomenclatural instability that is in fact avoidable. A law of conservation has often been advocated, but experience has tended to show that the considerations involved in a decision as to whether the normal provisions of the Code should be suspended in any given case are too complex to be covered adequately be any rigid rule of this kind. Two alternative courses have been tried in recent years. The first has been to permit certain specified contraventions of the normal provisions of the Code, after suitable notice has been published, subject to the right of any zoologist to object to the contravention within a specified period—in which case the matter goes to the International Commission. This course has been very little availed of. The second course is to make use of the plenary powers of the Commission to determine individual cases under suspension of the Code. This has been followed extensively, and in consequence the Commission has been able to clear up a great number of very refractory problems during the last ten years.

If the Commission is to be used by zoologists on this scale, and if it is to be in a position to meet reasonably expeditiously the demands made upon it, then it will be necessary to ensure that it has at its disposal adequate facilities and staff—and that means adequate finance. The Commission's activities are financed solely through the sale of its publications, and the Fifteenth International Congress of Zoology resolved that steps should be taken to reduce drastically the price of those publications. How in these circumstances the Commission is to be assured of an income adequate for the maintenance of its activities at their past level—let alone for their expansion—I, for one, am unable to see.

VAN DER VECHT, J., Leiden: The attention of taxonomists is drawn to Lichtenstein's "Catalogus musei Zoologici ditissimi Hamburgi" (1796) which contains about 500 new species in various groups of insects. A photocopy of this rare work will be shown. Lichtenstein's names have been overlooked by most later authors, but since they are evidently validly published, special steps must be taken if it would be regarded desirable to reject these names. It is shown that introduction of these names as well as their rejection would raise nomenclatorial problems. Specialists are therefore requested to cooperate in an attempt to determine the consequences of the acceptance of the Lichtenstein names in different groups of insects.

D. POVOLNY, Brno: 1. Es steht fest, daß die pedantische Anwendung des Prioritätsprinzips in der Vergangenheit zu großer Verwirrung der zoologischen und vor allem der entomologischen Nomenklatur geführt hat. Formalistische Tendenzen, welche dadurch entstanden sind, führten zur Entwicklung einer Art von Entomologen, welche jede Verbindung mit der konkreten Wissenschaft verloren und den Sinn ihrer Tätigkeit in Ausgrabungen alter Namen und in der Lösung rein formalistischer Fragen gesehen haben. Dadurch haben sie sich von der konkreten Wissenschaft selbst isoliert.

2. Das Bewußtsein einer formalistischen Gefahr besteht aber auch in der ICZN. Diese hat eine schwierige Entwicklung durchgemacht, trotzdem aber große Arbeit geleistet, wie auch die letzten Erfolge zeigen. Die durch den Internationalen Zoologenkongreß in London 1958 eingeführten „Nomina oblita" sind ein Beweis dafür, daß die ICZN offenbar ihre Aufgabe nach wie vor gut kennt und löst.

3. Erzwingung einer Stabilität der Taxonomeninhalte ist Kampf gegen wissenschaftliche Erkenntnisse und Forschung in der Zoologie und muß auf das strengste verurteilt werden.

4. Die Namensänderungen, welche aus dem wissenschaftlichen Fortschritt auf dem Gebiete der Taxonomie resultieren, müssen auch weiterhin als gerechtfertigt angesehen werden.

5. Nach Teil IV der Erläuterungen zum Einladungsschreiben für das Symposium gehen die Hauptziele des Symposiums am Aufgabenbereich der Internationalen Nomenklaturkommission vorbei. Diese Ziele stehen im auffallenden Widerspruch zum Titel des Symposiums: „Grundfragen der Systematik und Nomenklatur".

6. Die Autorität der ICZN muß weiterhin durch internationale Zusammenarbeit der Zoologen gefördert werden. Deshalb muß der Sinn nichtoffizieller Beratungen, Symposien etc. vor allem in der Aufgabe liegen, konstruktive Motive, Entwürfe und Gedanken der ICZN zur Verfügung zu stellen. Jede Tendenz, eine destruktive Tätigkeit gegen die ICZN zu organisieren, mag sie auch durch gelehrte Definitionen usw. getarnt werden, muß bekämpft werden.

L. B. HOLTHUIS, Washington: As far as the question about the relation between classification and nomenclature is concerned, these in my opinion are two totally different entities. Classification, or taxonomy, is a living science, nomenclature is a technical tool, be it an important

tool used extensively in taxonomy. Nomenclature can be defined as the action of giving names (in this case to zoological taxa). The nomenclatural rules which govern the naming of these taxa are men-made and therefore can be augmented, changed, and in some cases suspended by a group of individuals, the International Commission on Zoological Nomenclature, appointed for this purpose. In no way, however, should the Commission be given the authority to decide on taxonomic problems. Taxonomy, as I said before, is a living science; the laws and rules governing it have to be discovered and studied by the scientists, they cannot be made by them. A view on the history of taxonomic zoology clearly shows that it is impossible to tie taxonomy down by rules without halting its progress. Many taxonomic concepts which at some period were generally accepted by zoologists, later were proved to be wholly erroneous, and even this later conception might be emended still later.

As long as taxonomy is a living science its nomenclature cannot be perfectly stable; when that point is reached taxonomy will be dead and no longer a science. As long as there are "splitters" and "lumpers" in zoology, taxa will continue to be indicated with different names. We cannot dictate for instance that the correct name of the lion is *Felis leo*, and thus compel zoologists who have the conviction that the genus *Leo* is distinct from the genus *Felis* to change their taxonomic viewpoint; this would be an intolerable violation of the freedom of taxonomic thought. Nomenclatural changes reflecting differing taxonomic views may be unpleasant, but they are a sure sign that taxonomy is living and healthy, and I believe that no valid objection can be raised to this kind of nomenclatural instability. It is a dynamic change similar to that found in all growing sciences.

Something quite different, however, is the instability caused by purely technical nomenclatural reasons. This instability is unnecessary and should be fought by every available means. As I see it, the task of the International Commission is in the first place to straighten out these purely nomenclatural problems. Such problems are not of a subjective nature—that is, they are not created by a valid difference of opinion among zoologists about the status of a taxon, but they are objective since they are caused by a violation, incorrect application or wrong interpretation of the International Code of Zoological Nomenclature. If a conscientious Commissioner had to decide a taxonomic problem, he could not do so in good faith without being himself a specialist in the group concerned and without being able to fully evaluate the arguments pro and con the decision he is asked to make, and even then he must be aware that what he now thinks to be the correct solution may be rejected in years to come by the majority of zoologists. In cases of pure nomenclature on the other hand, any Commissioner, whatever his speciality or interest, as long as he is the specialist in nomenclature that he is expected to be, can decide the proper course to be followed under the terms of the International Code. For this he does not need to be a specialist in the group, nor does he need the advice of such a specialist. He only needs the help of specialists to get all the data concerning a certain case, so that he may give a well-founded opinion. Furthermore in cases where the suspension of the Rules is requested, he needs to know the general feeling about such a suspension among specialists and non-specialists. Otherwise, however, he can decide the merits of a certain case objectively, even without knowing what the animals concerned look like.

Summarizing I may state that the Commission is a body of specialists in nomenclature and therefore capable to make decisions on purely nomenclatural matters, but that neither it nor any other body of zoologists is competent to make binding decisions an taxonomic problems without infringing on the freedom of taxonomic thought.

S. WAGENER: Wagener schlug vor, in das Programm des nächsten Internationalen Entomologenkongresses von vornherein ein Symposium über ,,Grundlagen der Systematik und Nomenklatur" einzuplanen. Bis dahin hätten die Teilnehmer Zeit, sich mit den angeschnittenen Fragen auseinanderzusetzen, sie reifen zu lassen und unter Umständen neue Gesichtspunkte oder auch Wege zu einer Lösung zu ermitteln.

Die Stimmen des Symposiums waren dafür. Herr Martini schlug sogar vor, lieber noch ein kleines Symposion zwischenzuschalten, um die Aussprache in Fluß zu halten. Auch dieser Gedanke gefiel.

Es wurde angeregt, den begonnenen Gedankenaustausch über grundsätzliche Fragen auch in bestimmter literarischer Form fortzusetzen. Über einige Worte der Gestaltung kam die Erörterung nicht hinaus, diese bleibt der Zukunft überlassen (z. B. zunächst in Form von Diskussionsbriefen zwischen den speziell interessierten Fachgenossen, die zentral redigiert werden).

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XI. INTERNATIONALER KONGRESS FÜR ENTOMOLOGIE

VIEN 1960

VERHANDLUNGEN

BAND III

SYMPOSIUM 3: *Chemie der Insekten.*

SYMPOSIUM 4: *Chemische Verteidigungsmechanismen bei Arthropoden.*

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1960

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Präsident: Prof. Dr. K. E. Schedl - *Generalsekretär*: Dr. M. Beier

WIEN, 17 - 25 August 1960

VERHANDLUNGEN - Band III

SYMPOSIUM 3:

CHEMIE DER INSEKTEN
INSECT CHEMISTRY
CHIMICA DEGLI INSETTI
CHIMIE DES INSECTES

SYMPOSIUM 4:

CHEMISCHE VERTEIDIGUNGSMECHANISMEN BEI ARTHROPODEN
CHEMICAL DEFENSIVE MECHANISMS IN ARTHROPODS
MECCANISMI CHIMICI DI DIFESA NEGLI ARTROPODI
MECANISMES CHIMIQUES DE DEFENSE CHEZ LES ARTHROPODES

Edited by:

Prof. M. Pavan, Chairman of Symposium 3

and

Prof. T. Eisner, Chairman of Symposium 4.

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XI. INTERNATIONALER KONGRESS FÜR ENTOMOLOGIE, WIEN 1960

Präsident: Prof. Dr. K. E. Schedl - *Generalsekretär*: Dr. M. Beier

Symposium 3: CHEMIE DER INSEKTEN

Präsident: Prof. Dr. M. Pavan

Symposium 4: CHEMISCHE VERTEIDIGUNGSMECHANISMEN BEI ARTHROPODEN

Präsident: Prof. Dr. T. Eisner

Die Präsidenten und die Teilnehmer am Symposium « Chemie der Insekten » und am Symposium « Chemische Verteidigungsmechanismen bei Arthropoden » sowie der Präsident und der Generalsekretär des XI. Internationalen Entomologenkongresses, sagen ihren herzlichen Dank an den:

— *Herrn Ministerpräsident Italiens*: Prof. Dr. A. Fanfani
— *Herrn Unterrichtsminister*: Prof. Dr. G. Bosco
— *Herrn Rektor der Universität Pavia*: Prof. Dr. L. De Caro

die dem Institut für die Agrarentomologie der Universität Pavia die Finanzierung für die Herausgabe des vorliegenden Buches gewährt haben, wodurch die sofortige Veröffentlichung der wissenschaftlichen Resultate der zwei Symposia ermöglicht wurde.

Prof. Mario Pavan
Vorstand des Istituto di Entomologia Agraria dell'Università di Pavia

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XIth INTERNATIONAL CONGRESS OF ENTOMOLOGY, VIENNA 1960

President: Prof. Dr. K. E. Schedl - *General Secretary*: Dr. M. Beier

Symposium 3: INSECT CHEMISTRY

Chairman: Prof. Dr. M. Pavan

Symposium 4: CHEMICAL DEFENSIVE MECHANISMS IN ARTHROPODS

Chairman: Prof. Dr. T. Eisner

The Chairmen and those taking part in the Symposium on « Insect Chemistry » and the Symposium on « Chemical Defensive Mechanisms in Arthropods », as well as the President and the General Secretary of XIth International Congress of Entomology, tank:

— *The Premier of the Italian Government*: Prof. A. Fanfani
— *The Minister of Education*: Prof. G. Bosco
— *The Rector of Pavia University*: Prof. L. De Caro

who have placed at the disposal of the Institute of Agrarian Entomology of Pavia University, the funds for the printing of the present volume, thus rendering possible the prompt publication of the results of the two Symposia.

Prof. Mario Pavan
Director of Istituto di Entomologia Agraria dell' Università di Pavia

XI CONGRESSO INTERNAZIONALE DI ENTOMOLOGIA, VIENNA 1960

Presidente: Prof. Dr. K. E. Schedl - *Segretario Generale*: Dr. M. Beier

Simposio 3: CHIMICA DEGLI INSETTI
Presidente: Prof. Dr. M. Pavan

Simposio 4: MECCANISMI CHIMICI DI DIFESA NEGLI ARTROPODI
Presidente: Prof. Dr. T. Eisner

I Presidenti e i partecipanti al Simposio « Chimica degli Insetti » e al Simposio « Meccanismi chimici di difesa negli Artropodi », il Presidente ed il Segretario Generale dell' XI Congresso Internazionale di Entomologia, ringraziano:

— *il Presidente del Consiglio dei Ministri d' Italia*: prof. A. Fanfani
— *il Ministro della Pubblica Istruzione*: prof. G. Bosco
— *il Rettore dell' Università di Pavia*: prof. L. De Caro

che hanno messo a disposizione dell' Istituto di Entomologia Agraria dell' Università di Pavia il finanziamento per la stampa del presente volume, consentendo così l'immediata pubblicazione dei risultati dei due Simposi.

Prof. Mario Pavan
Direttore dell' Istituto di Entomologia Agraria dell' Università di Pavia

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XI^e CONGRES INTERNATIONAL D' ENTOMOLOGIE, VIENNE 1960

Président: Prof. Dr. K. E. Schedl - *Secrétaire Général*: Dr. M. Beier

Symposium 3: CHIMIE DES INSECTES
Président: Prof. Dr. M. Pavan

Symposium 4: MECANISMES CHIMIQUES DE DEFENSE CHEZ LES ARTHROPODES
Président: Prof. Dr. T. Eisner

Les Présidents et tous ceux qui ont participé au Symposium « Chimie des Insectes » et au Symposium « Mécanismes chimiques de défense chez les Arthropodes » ainsi que le Président et le Secrétaire général du XI^e Congrès International d' Entomologie, remercient:

— *Le Président du Conseil des Ministres d' Italie*: Prof. A. Fanfani
— *Le Ministre de l' Instruction Publique*: Prof. G. Bosco
— *Le Recteur de l' Université de Pavie*: Prof. L. De Caro

qui ont mis à la disposition de l' Institut d' Entomologie Agraire de l' Université de Pavie les moyens financiers nécessaires pour faire imprimer ce volume, nous permettant ainsi de publier immédiatement les comptes rendus des deux Symposia.

Prof. Mario Pavan
Directeur de l' Istituto di Entomologia Agraria dell' Università di Pavia

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SYMPOSIUM 3 :

INSECT CHEMISTRY
CHEMIE DER INSEKTEN

CHIMICA DEGLI INSETTI
CHIMIE DES INSECTES

Symposium 3: INSECT CHEMISTRY

Vienna, August 22, 1960

INTRODUCTION

In 1958 there was held in Vienna, in connection with the 4th International Congress of Biochemistry, a special Symposium on insect biochemistry, organized by Dr. L. Levenbook. Twenty-four authors contributed a total of 16 papers, dealing with topics that were selected by the organization of the Symposium. The contributors were primarily biochemists. Their papers have been published collectively in a volume of fundamental interest (1).

The studies involving chemistry and insects are continuously increasing in general significance. Their prospects for development are truly remarkable, due in no small measure to the increasing participation of outstanding scientists and internationally renown institutes.

These considerations justify our having introduced to the organizers of the 11th International Congress of Entomology the proposition that if biochemists have felt the need for a Symposium on insect biochemistry, then surely entomologists should call attention to developments in this sector which is really a branch of entomology. The Congress organization urged me to accept this task, even though I felt that other entomologists might better be chosen to do it.

I have proposed that the first Symposium on insect chemistry might aim at:

1) fostering the widest contact between chemists of all kinds and entomologists, thus rendering easier the synthesis of their viewpoints and methods from which we expect benefits for science and for humanity;

2) encouraging the widest participation of scientists by inviting communications on any topic of research involving in some way chemistry and insects.

We have therefore limited general discussion to a single opening lecture by Professor Thomson on a topic that touches directly or indirectly on many important points in our field of investigation.

I have invited 170 noted scientists to participate in the Symposium, with the result that 53 authors are presenting 39 communications. We should have

(1) IV International Congress of Biochemistry, 1958; Vol. XII: Biochemistry of Insects; Pergamon Press, London, 1959.

had a greater number if had been possible to pay costs of travel, in many cases quite expensive.

We are now gathered to present the latest results of our studies, to evaluate the possibilities of the work, and to learn how to develop it further. Apart from the contacts made among those of us who are interested in the arguments of our communications, it will be important too to join in discussion with all other entomologists, for it is from entomologists that we must get the first indications of facts that need investigation by our methods, as well as information needed in order to carry out that investigation. Since insects are the objects of all this, no occasion could be more suitable and richer in possibilities than this one afforded by the International Congress of Entomology.

The list of the authors and titles of the papers which appear in the official programme of the Congress have been slightly altered: for example some participants presented their reports in other sections of the Congress and others have not delivered their reports at all.

The large number of papers submitted to the Symposium would have necessitated at least a three day meeting of delivery and discussion and owing to reasons of organisation we had to accept the time limit, as was forseen in the invitation circular, of only 10 minutes for each report and cancel the general discussions.

The remarkable number of papers at this Symposium, the variety of the fields of research dealt with, the general importance of the research concerning « insect-chemistry », the interesting prospects of work that it opens up, confirm the opportuneness of the proposition to the committee of the XIth International Congress of Entomology of including, as an experiment, the Ist Symposium of Insect Chemistry.

I can already say with confidence that our Symposium will furnish material for a very interesting volume. Therefore we hope that this Symposium is the beginning of a new tradition of Symposia on Insect Chemistry in the International Congresses of Entomology.

I thank all those present and those who have given this Symposium the results of their studies; on behalf of you all I thank the organization of the Congress that has accepted us.

Therefore I open the proceedings of the First Symposium on Insect Chemistry, and wish you all great success and new fruitful developments of your activities.

Mario PAVAN
Istituto di Entomologia Agraria
dell' Università di Pavia (Italia)

INTRODUZIONE

Nel 1958 a Vienna si è tenuto un Simposio di biochimica degli Insetti, organizzato dal Dr. L. Levenbook per il IV Congresso Internazionale di biochimica, al quale 24 Autori hanno presentato 16 relazioni su temi stabiliti dall'organizzazione del Simposio. Tale Simposio fu caratterizzato dalla partecipazione prevalente di biochimici. Le relazioni sono state raccolte in un volume di interesse fondamentale (1).

Gli studi imperniati sulla chimica e sugli Insetti vanno continuamente aumentando di profondità, di estensione e di importanza generale; le prospettive di sviluppo che essi presentano sono veramente notevoli anche per il fatto che di essi si occupano a fondo scienziati e scuole di rinomanza internazionale.

Questi fatti e considerazioni ci giustificano nell'aver richiamato l'attenzione dell'organizzazione dell'XI Congresso Internazionale di Entomologia sull'opportunità che come i biochimici hanno sentito il bisogno di fare un Simposio sulla biochimica degli Insetti, così gli entomologi raccolgano l'attenzione sugli sviluppi di questo settore che ha nell'entomologia la sua base essenziale. L'organizzazione del Congresso ha voluto con insistenza che me ne occupassi nonostante il mio desiderio che altri studiosi fossero incaricati di questo compito. Ho proposto così che il I Simposio di chimica degli Insetti fosse imperniato sui seguenti concetti:

— favorire il più largo contatto fra i chimici di ogni tendenza e gli entomologi per facilitare il realizzarsi di quelle fusioni ed integrazioni delle rispettive tendenze e possibilità di lavoro dalle quali vi è da attendersi benefici per tutti e per la scienza.

— aprire perciò la partecipazione al più largo numero possibile di studiosi mediante la possibilità di presentare comunicazioni su qualsiasi tema di ricerca nella quale siano coinvolti in qualche modo la chimica e gli Insetti.

Abbiamo così limitato le trattazioni generali ad una sola conferenza iniziale del prof. Thomson su un tema che tocca direttamente e indirettamente molti importanti settori del nostro campo di lavoro.

Con tali prospettive ho invitato a partecipare al Simposio 170 studiosi a me noti: come risultato abbiamo avuto l'iscrizione di 53 Autori con la presentazione di 39 comunicazioni.

Avremmo avuto maggiore afflusso se fosse stato possibile distribuire sussidi per le spese di viaggio che in certi casi sono veramente ingenti.

Ci troviamo ora riuniti per presentare gli ultimi risultati dei nostri studi, per valutare le possibilità di lavoro, per trovare le vie per svilupparlo.

Oltre ai contatti fra coloro che si occupano già degli argomenti delle nostre comunicazioni, avranno importanza i contatti con gli entomologi in generale dai quali possono venire fornite indicazioni di fenomeni da indagare con le nostre metodiche e su possibilità di affrontarli. Poichè gli Insetti sono la base di tutto ciò, nessuna occasione poteva essere più opportuna e ricca di possibilità di quella offerta dal Congresso Internazionale di Entomologia di cui siamo membri.

L'elenco degli Autori e i titoli delle comunicazioni che appaiono nel « Programma » ufficiale del Congresso hanno subìto qualche variazione: ad esempio alcuni studiosi presen-

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(1) *IVth Int. Congress of Biochemistry*, 1958; vol. 12: *Bioch. of Insects*; Pergamon Press. London, 1959.

tano le loro relazioni in altra sede del Congresso, mentre altri Autori hanno rinunciato a presentare le loro relazioni.

Il grande numero di comunicazioni iscritte al Simposio avrebbe richiesto almeno tre giorni di riunione per l'esposizione e le discussioni ma per ragioni organizzative abbiamo dovuto accettare la limitazione di tempo, come era previsto nella circolare di invito, a soli 10 minuti per ciascuna comunicazione e sopprimere necessariamente le discussioni pubbliche.

Il notevole afflusso di comunicazioni a questo Simposio, la varietà dei campi di lavoro in esso trattati, la generale serietà e importanza delle ricerche svolte attorno al binomio «chimica - insetti», le interessanti prospettive di lavoro che esse aprono, confermano l'opportunità di aver proposto all'organizzazione dell' XI Congresso Internazionale di Entomologia di accogliere in via sperimentale il I Simposio di Chimica degli Insetti.

Il nostro Simposio fornirà materiale per un volume molto interessante: appare perciò augurabile che il nostro Simposio segni l'inizio di una tradizione nuova dei Simposi di chimica degli Insetti nei Congressi Internazionali di Entomologia.

Ringrazio ora tutti i partecipanti che hanno voluto portare a questo Simposio il frutto dei loro studi e ringrazio l'organizzazione del Congresso che ci ha accolto permettendo la realizzazione di questo primo positivo esperimento.

Apro perciò i lavori del I Simposio di Chimica degli Insetti augurando a tutti buon successo e nuovi fruttuosi sviluppi delle loro attività.

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THOMSON R H. (*)

INSECT PIGMENTS

Chemists have always been interested in natural colouring matters. Their investigations on plant pigments have been highly successful and the chemical structures of several hundreds are fully established. By comparison, our knowledge of insect pigments is very limited and relatively few (less than thirty) have been completely identified. The difficulty of obtaining adequate amounts of material for investigation, coupled with the intractable nature of many insect pigments, is largely responsible for this, but considerable advances have been made in recent years using modern chromatographic and spectrometric techniques. It is not possible in this brief review to discuss the chemistry of insect colouring matters in any detail, but I should like to direct attention to the origin and biogenesis of these substances, and to the problems requiring further investigation.

The following groups of compounds appear to be responsible for most of the pigmentary (as distinct from structural) colouring in insects.

Pteridines	—	white, yellow, orange, red.
Ommochromes	—	yellow, red, brown.
Carotenoids	—	yellow, orange, red, blue (green – insectoverdins).
Quinones	—	yellow, red.
Melanins	—	brown, black.

They can be classified, in terms of their origin, into three groups:

(a) Endogenous pigments formed by metabolism of amino-acids – pteridines, ommochromes, melanins.

(b) Exogenous pigments derived from plants – flavones, carotenoids (insectoverdins).

(c) Pigments of uncertain origin, possibly biosynthesised by symbiotic microorganisms – anthraquinones, aphins.

I propose to deal with each group in turn, leaving the endogenous pigments to the last.

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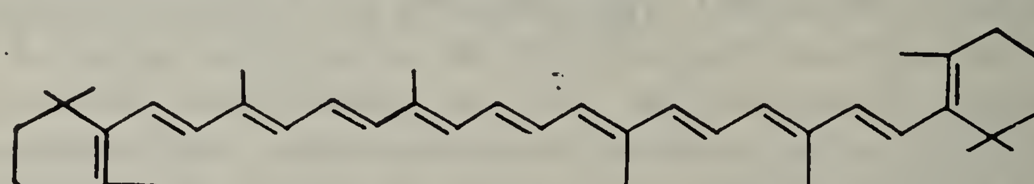
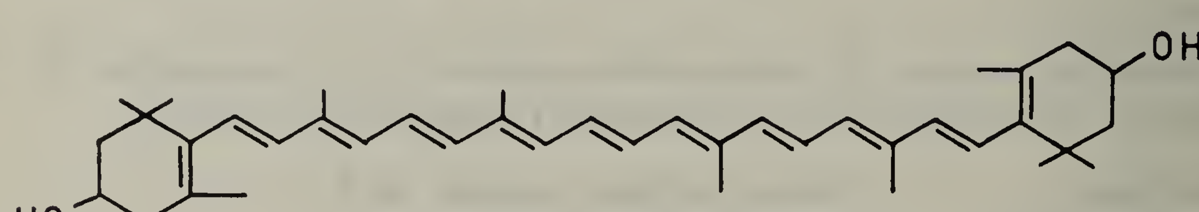
EXOGENOUS PIGMENTS

Flavones and carotenoids are very widely distributed in plants and hence are commonly present in the diet of phytophagous animals. After ingestion they may be excreted, degraded to colourless products or stored, either indiscriminately or selectively. The pigments retained may accumulate unchanged, or undergo chemical modification. All these possibilities have been observed with carotenoids but there is little data as yet on flavones.

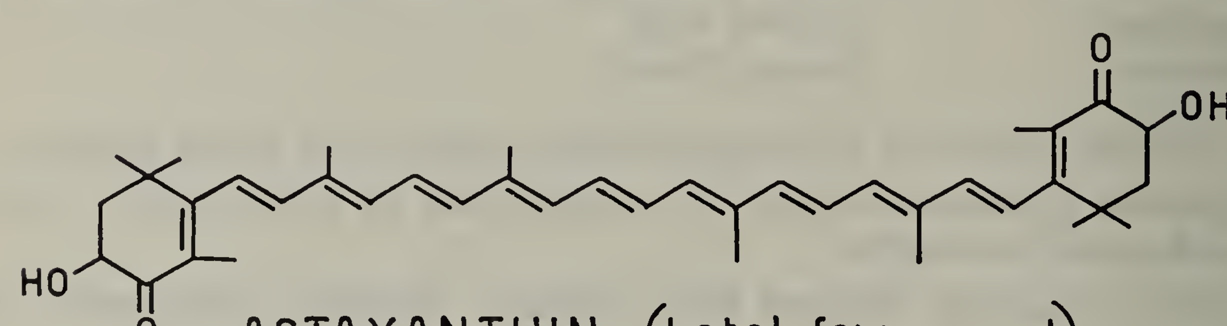
Carotenoids. These have been detected in numerous insects, both in the free state and combined as water-soluble protein complexes. They occur as wing pigments, in haemolymph, integument, and other tissues, and also in beeswax and propolis (derived from pollen). It is of interest that carotenoids are not confined to phytophagous insects but are also present in certain predaceous species, such as the bug *Perillus bioculatus* (1) which preys on the potato beetle *Leptinotarsa decemlineata*, and in the mite *Microgaster conglomeratus* (2) which



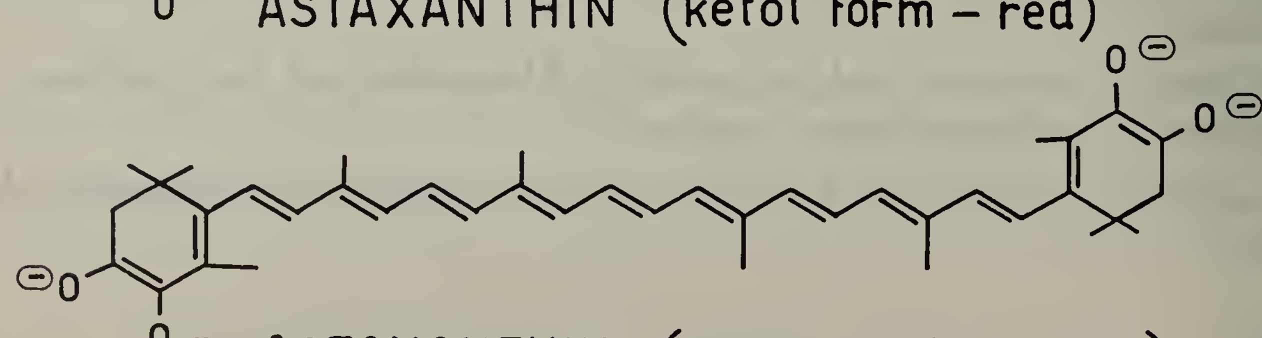
β-CAROTENE (orange-red)



LUTEIN (orange-red)



ASTAXANTHIN (ketol form – red)

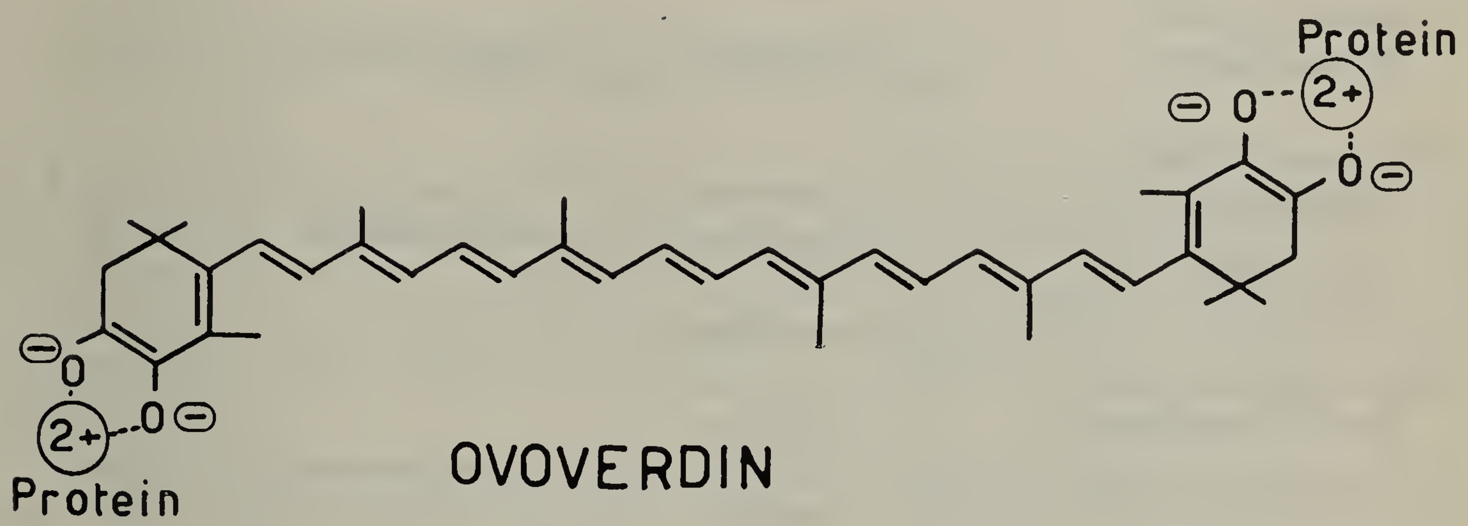


ASTAXANTHIN (enediol anion – blue)

infests *Pieris brassicae.* β-Carotene probably occurs in all green leaves, as well as many flowers and fruits, and is consequently the carotenoid most commonly encountered in insects (3). α-Carotene which occurs fairly frequently with the β-isomer in leaves, has also been reported several times e.g., in *Coccinellidae* (4). Of the xanthophylls (oxygenated carotenoids), lutein is the commonest in higher plants and probably also in insects. It has been isolated in crystalline form by Oku (5) from the cocoon of *Bombyx* mori and also from the mulberry leaves on which it feeds.

The most interesting xanthophyll is astaxanthin which is responsible for the red and blue colouration of some *Orthoptera.* This pigment is not found in higher plants (*) but occurs frequently in the *Crustacea.* The stable ketol structure is red, like most carotenoids, but the enediol tautomer forms blue salts.

Kuhn and Sörensen (6) concluded that ovoverdin, the blue-green chromoprotein in the shell of the lobster, is an enol salt of this type, and Okay (7) has shown that a similar astaxanthin-protein complex forms the blue pigment



OVOVERDIN

in the wings of the locusts *Oedipoda coerulescens* and *Oe. schochii.* The red wings of *Oe. miniata* and other spp. also contain an astaxanthin-protein complex, the pigment being in the ketol form presumably, and Okay found that even the yellow chromoprotein in the wings of *Oe. aurea* decomposed in chloroform liberating a red carotenoid which was apparently astaxanthin. The same pigment is present in the eyes, wings and integument of *Locusta migratoria,* occurring as an enol chromoprotein in atypical blue specimens (8). Astaxanthin is not present in the food of the locust and is most probably derived from β-carotene (rather than from xanthophylls) by an oxidative process. This view is supported by Goodwin's observation (8) that the β-carotene present in newly laid *Locusta* and *Schistocerca* eggs disappears during incubation and is largely

(*) Esterified astaxanthin was found recently in the petals of *Adonis annua* L. (*Ranunculaceae*) (A. Seybold and T. W. Goodwin, *Nature* (London), 1959, 184, 1714-5). This is the first record of its occurrence in higher plants.

replaced by astaxanthin. Thus locusts provide a good example of the selective accumulation and modification of a single dietary carotenoid, and the economic utilisation of one pigment (β-carotene) for the production of a range of colours. The mechanism of the β-carotene ——> astaxanthin conversion merits investigation.

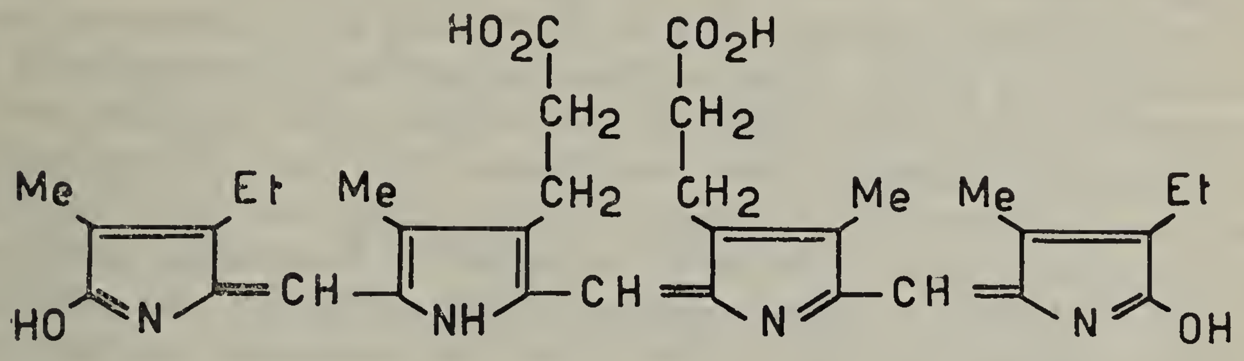
INSECTOVERDINS. Although chlorophyll is of universal distribution in higher plants, the occurrence of green pigments in animals is rare and green colouration, apart from that due to iridescence, normally arises from a combination of yellow and blue pigments [or yellow pigment + Tyndal blue (formed

TABLE 1.
TYPICAL INSECTOVERDINS

Species	Pigment Components		Ref.
	Yellow	Blue	
Carausius morosus (haemolymph)	β-carotene	mesobiliverdin	11
Schistocerca solitaria (haemolymph)	β-carotene		
(integument)	β-carotene + astaxanthin	mesobiliverdin	12
Pieris rapae (larval haemolymph)	β-carotene + lutein	mesobiliverdin	10
Cacoecia australana (larval haemolymph)	β-carotene + lutein	mesobiliverdin	10
Plusia gamma (larval haemolymph)	lutein	mesobiliverdin	13
Papillo xanthus (pupal cuticle)	β-carotene + lutein + ?	mesobiliverdin	14

by light scattering) e.g., in green feathers (9)]. An insectoverdin is a green pigment complex consisting of yellow and blue chromoproteins. They occur particularly in the integument and haemolymph of *Orthoptera* and *Lepidoptera* but

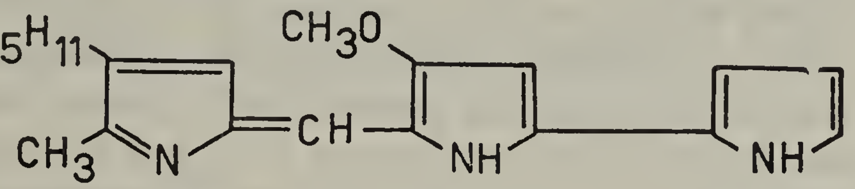
may be fairly widely distributed. In a typical case the yellow component is a carotenoid and the blue component is a bile pigment (see Table 1) but other combinations have been observed, e.g., in the Pentatomid bug *Nezara viridula* the blue component is an unidentified anthocyanin-like pigment (10). The chief problem connected with the insectoverdins concerns the origin and structure of the so-called, bile pigments (linear tetrapyrroles). It is fairly certain, from their spectra and colour reactions, that they are all bilitrienes of the mesobiliverdin



MESOBILIVERDIN

type, but it cannot be assumed that they are all identical. This can only be established by further studies as exemplified by those of Wieland (15) on the blue chromoproteins of Pierid butterfly wings. The blue pigment from *Pieris brassicae*, isolated in the form of its crystalline dimethyl ester, appears to be a mesobiliverdin, and it should be possible to establish its structure by modern methods (16).

Virtually nothing is known concerning the origin of these tetrapyrrole pigments. Although *Rhodnius prolixus* and other blood-sucking *Hemiptera* (17) can degrade haemoglobin to biliverdin, and phytophagous Hemipterous bugs [e.g., *Anasa tristis* (18)] break down chlorophyll to a green pigment which gives



PRODIGIOSIN
(ex. Bacillus prodigiosus
= Serratia marcescens)

a positive Gmelin reaction, it is unlikely that insect bile pigments in general, are formed by porphyrin degradation, as in higher animals. Okay (19) has shown by feeding experiments, that the blue bile pigment in green *Orthoptera* is not a breakdown product of chlorophyll or haemoglobin. It seems much

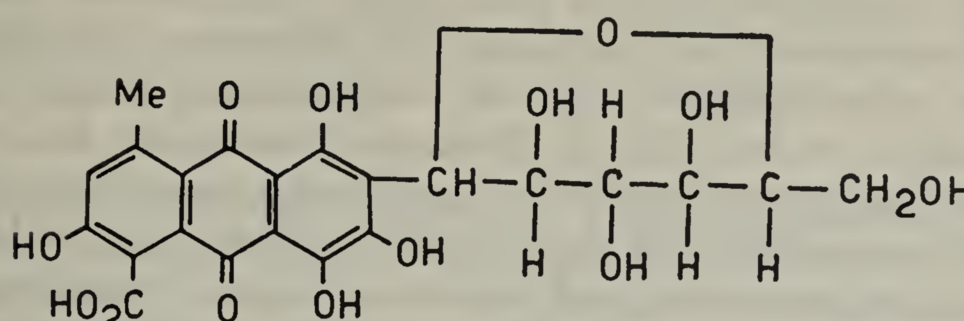
more probable that these pigments are built up *de novo* from glycine and acetate, like the porphyrins, possibly via proline derivatives which appears to be the case in the biogenesis of the linear tripyrrole pigment, prodigiosin (20), a metabolite of *Serratia marcescens.* If so, they fall into class (a), endogenous pigments formed by metabolism of amino-acids, where no doubt they will appear in a future review.

FLAVONES. Despite the abundance of this type of pigment in the food of phytophagous insects there are very few reports of the occurrence of flavones in these, or indeed in any animals, and in most cases the « evidence » is limited to the formation of a yellow colour in alkaline solution. Nevertheless it is likely that flavones do occur fairly widely in low concentration but they are masked by other pigments. Although their contribution to insect colouration is small they may have considerable chemical interest. Only two have actually been isolated. From the wings of the Satyrine butterfly *Melanargia galathea,* D. L. Thomson (21) obtained a yellow pigment which, although not identified, was undoubtedly a flavone. He also showed that this substance was present in the food plant (*Dactylis glomerata*) of the larvae. A more intriguing find is that of Oku (22) who isolated a flavone-like compound, bombycin, from the green cocoons of *Bombyx mori.* On hydrolysis this afforded glucose and an aglycone, bombycetin, $C_{20}H_{19}NO_7$, having an absorption spectrum like that of quercetin. (Quercetin-3-glucoside was also isolated from mulberry leaves, the food of *Bombyx mori*). Unfortunately this unique amino-flavone has not been examined further but recent Japanese work (23) has revealed the presence of several flavonoid compounds in the green cocoons of *Bombyx mori*, one of which is presumably bombycin. The same group of pigments is present also in the cocoon of *Theophila mandarina* but they are not the same as those detected in mulberry leaves. This suggests that the dietary flavones are modified by the insect before storage, in one case at least, by the introduction of nitrogen. It is tempting to speculate on the structure of bombycetin. If it be assumed that it is closely related to quercetin we find that the molecular formulae of the pigments differ by a fragment C_5H_9N, which at once suggests the presence of an isopentenyl (prenyl) group, $(CH_3)_2C = CHCH_2$—. Prenylation of aromatic compounds (including flavones) occurs frequently in plants, the C_5 group being attached either to carbon or oxygen. No examples are known in which prenylation has occurred on nitrogen but should this prove to be the case in bombycetin, it will not be the first time that a new type of structure has been found initially in an insect pigment. This of course is mere speculation, but the possibility that insects accumulate flavones of unusual structure should encourage further investigation.

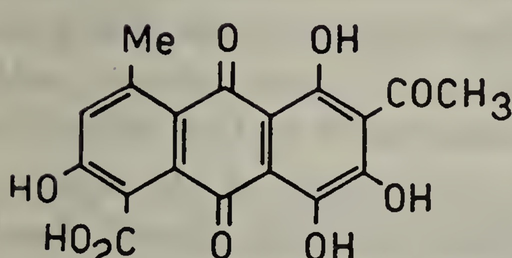
PIGMENTS OF UNCERTAIN ORIGIN

QUINONES. These pigments (24) are not of great significance as colouring matters in insects but they are of interest insofar as polyhydroxyquinones are rare in the animal kingdom. The anthraquinones elaborated by various *Coccidea*

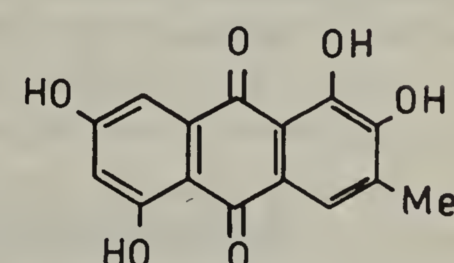
ANTHRAQUINONES IN COCCIDEA



CARMINIC ACID
(Dactylopius coccus)

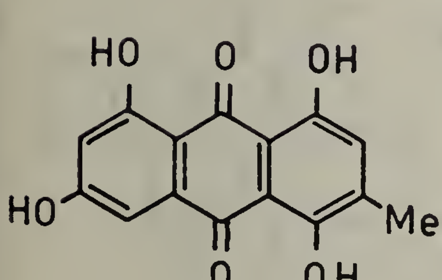


KERMESIC ACID
(Kermococcus ilicis)

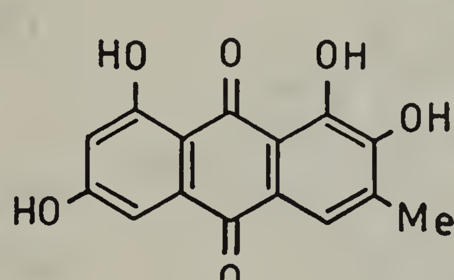


ERYTHROLACCIN
(Laccifer lacca)

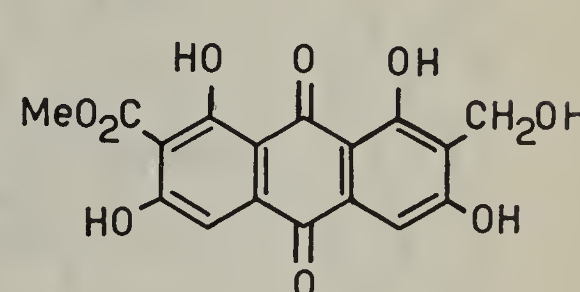
ANTHRAQUINONES IN PLANTS AND MICRO-ORGANISMS



CATENARIN
(Penicillium islandicum)



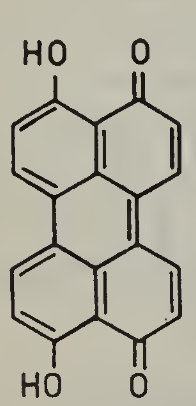
ALATERNIN
(Rhamnus Alaternus)



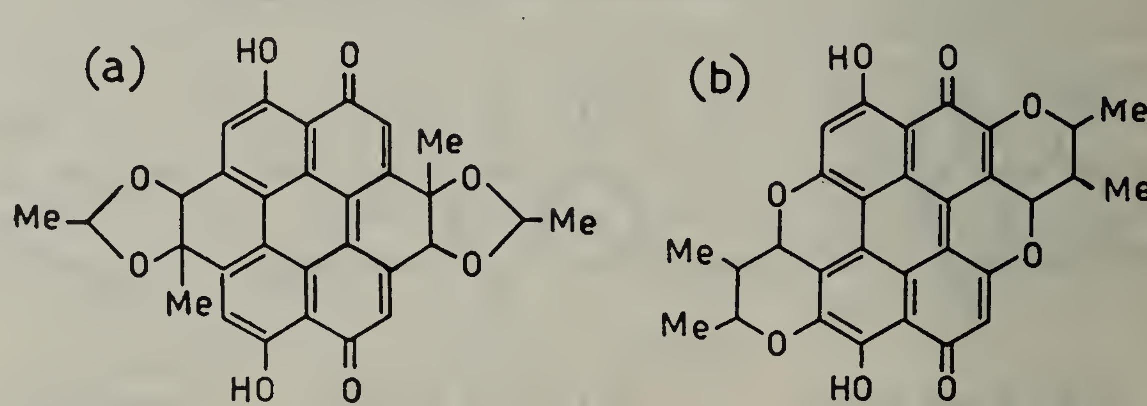
RHODOCLADONIC ACID
(Cladonia fimbriata)

were formerly of great importance as natural dyes; carminic acid, an unusual C-glycosyl compound, is the colouring principle of cochineal, and kermesic acid that of the ancient red dyestuff kermes. Laccaic acid, the pigment of lac dye, appears to be a mixture of quinones probably similar to kermesic acid. It is present in lac, the resinous secretion of *Laccifer lacca*, which also contains a minor quinonoid constituent, erythrolaccin (25). Numerous polyhydroxyanthraquinones occur in micro-organisms, lichens and flowering plants (see examples above) and the tracer studies (26) of Birch and Gatenbeck have conclusively established that the fungal anthraquinones are biosynthesised from acetate units. It is reasonable to assume that all the pigments of this type in the plant kingdom are formed in the same way, and inspection of the formulae of the coccid anthraquinones strongly suggests that these are also acetate-derived. However, there is no evidence as yet, which shows that this metabolic pathway operates in insects, nor indeed, is there any evidence so far, that animals can biosynthesise an aromatic ring *de novo* by any mechanism. This leads to the suggestion that these pigments may be built up from acetate by symbiotic micro-organisms. Much work has been done on symbiosis in *Coccidea* but not from this point of view (27). A study of this difficult problem would be of much value in relation to the whole question of the origin of aromatic compounds in the animal body.

Similar queries arise in connection with the biogenesis of the aphin pigments present in the haemolymph of many *Aphididae* and related families. Aphids ingest large quantities of plant juice, largely carbohydrate in content, some of which is probably converted into pigment by the route, sugar ——> acetate ——> protoaphin. The native pigments, protoaphins, are acidic, yellow, water-soluble, glucosides, which exist in the blood chiefly in the form of their deep red anions and are mainly responsible for the dark, almost black colour of many species. After death, the protoaphin undergoes a rapid enzymic conversion, in the presence of air, into three fat-soluble fluorescent pigments; the first step involves an oxidative hydrolysis, which is followed by a progressive



4, 9-DIHYDROXYPERYLENE-
- 3,10 -QUINONE



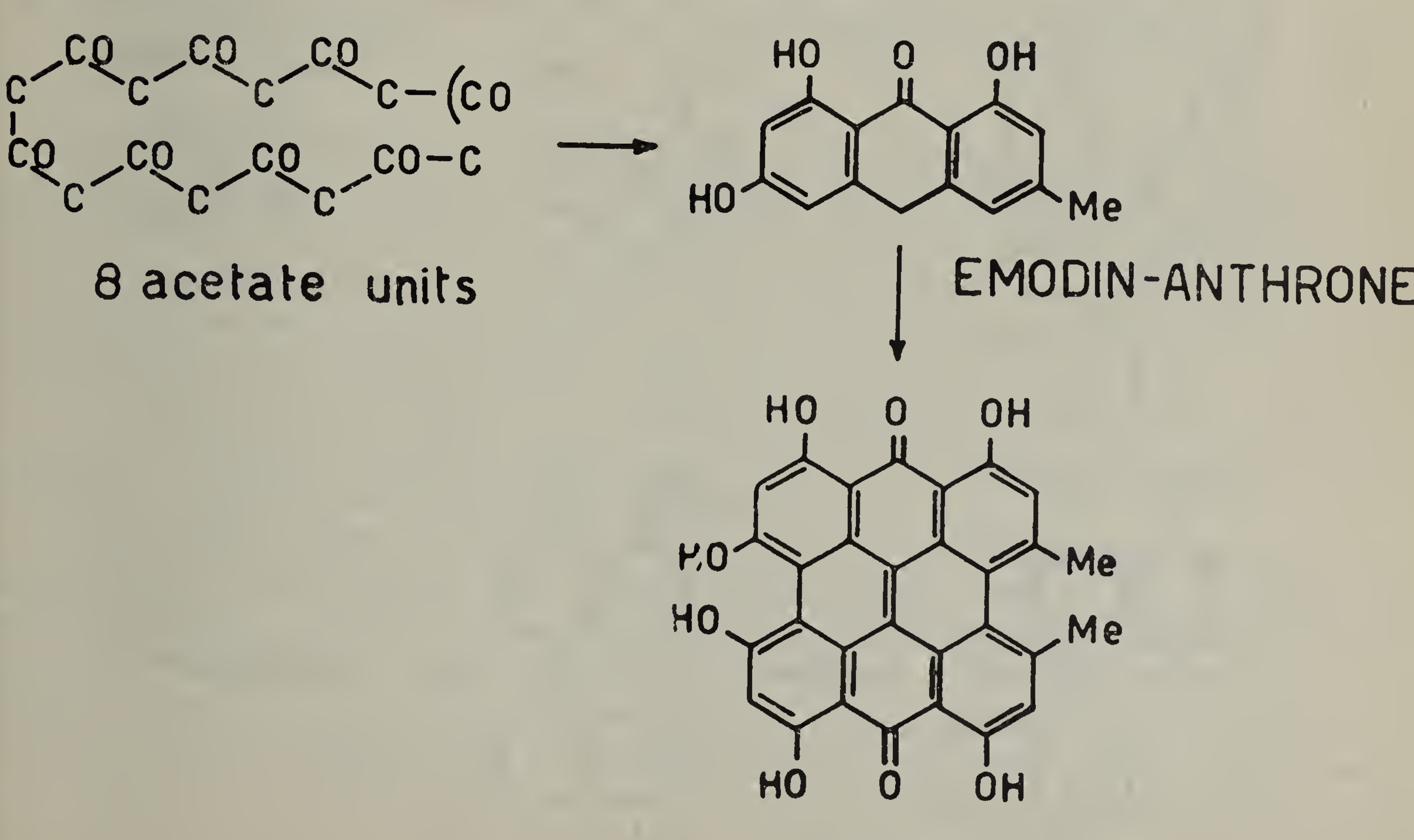
ERYTHROAPHIN
(two possible structures)

aromatisation of the xanthoaphin structure, a molecule of water being eliminated at each stage.

Protoaphin	——>	Xanthoaphin	——>	Chrysoaphin	——>	Erythroaphin
$C_{36}H_{40}O_{16}$		$C_{30}H_{26}O_{10}$		$C_{30}H_{24}O_{9}$		$C_{30}H_{22}O_{8}$
yellow		yellow		orange		red

The entire chemical investigation on these pigments has been carried out at Cambridge by Sir Alexander Todd and his collaborators. Little is known as yet concerning the structure of protoaphin, nor indeed whether the same pigment is present in all species, and most attention has been paid to the erythroaphins, the most stable artefacts. It has been established that the erythroaphin chromophore is 4,9-dihydroxyperylene-3,10-quinone but the structure of the remainder of the molecule is not yet settled. Two possibilities are shown above: biogenetic considerations lend weight to the prenylated structure (b). It is possible that protoaphin is a comparatively simple naphthalene derivative

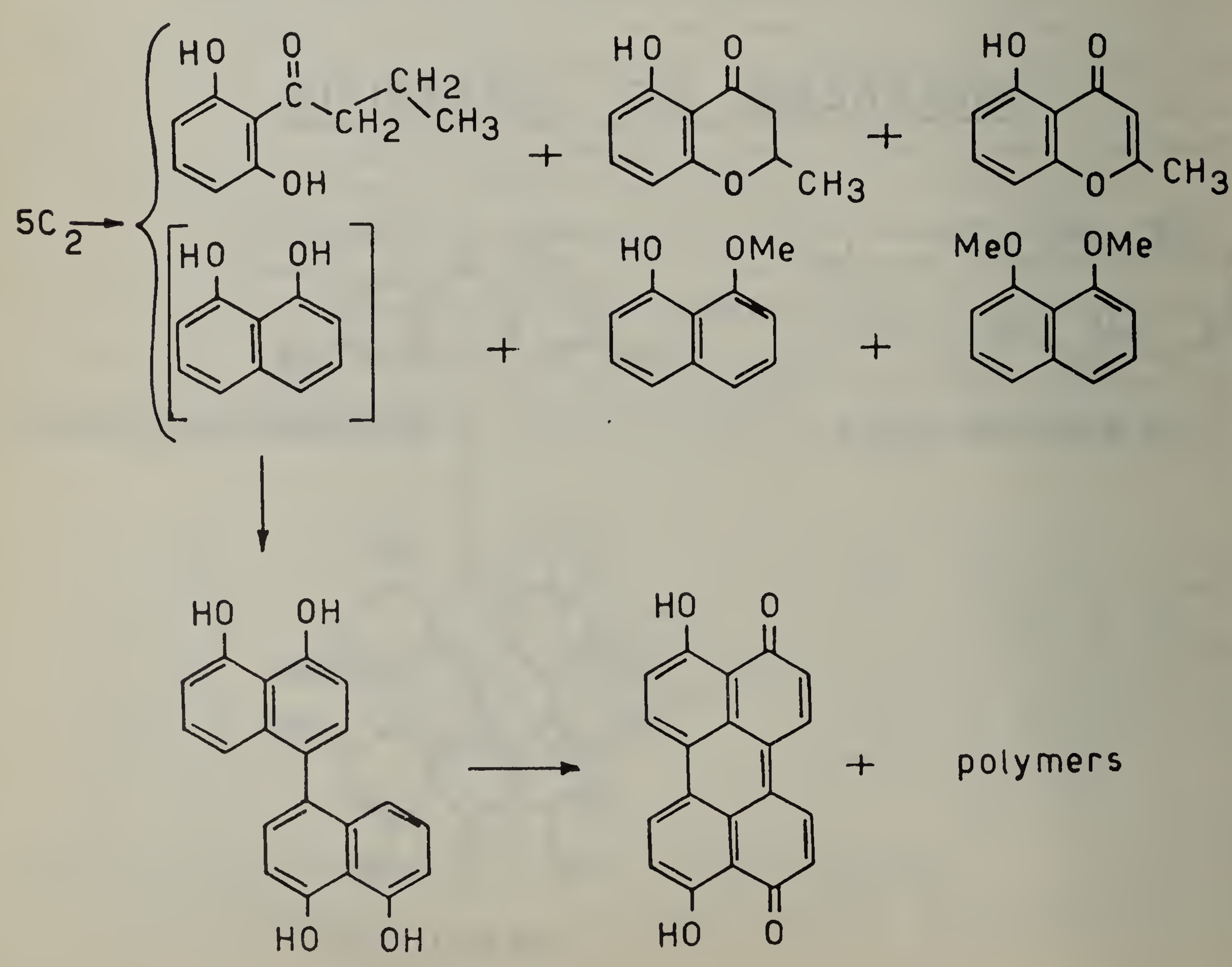
BIOGENESIS OF HYPERICIN



HYPERICIN

which undergoes oxidative dimerisation in a manner analogous to the formation of the complex pigment, hypericin, from emodin-anthrone, in plants of the *Hypericaceae* family (28). A close analogy is the formation of 4,9-dihydroxyperylene-3,10-quinone itself by the Ascomycete *Daldinia concentrica.* Using ^{14}C labelled acetate Bu' Lock (29) has been able to show that this fungus produces several C_{10} aromatic compounds by condensation of five C_2 units, including derivatives of 1,8-dihydroxynaphthalene. Enzymic oxidation of the latter leads to the formation of the quinone and other products. This of course is fungal metabolism. The aphin pigments may arise in a similar fashion but whether the protoaphins are authentic aphid metabolites or the products of symbiotic micro-organisms is an open question.

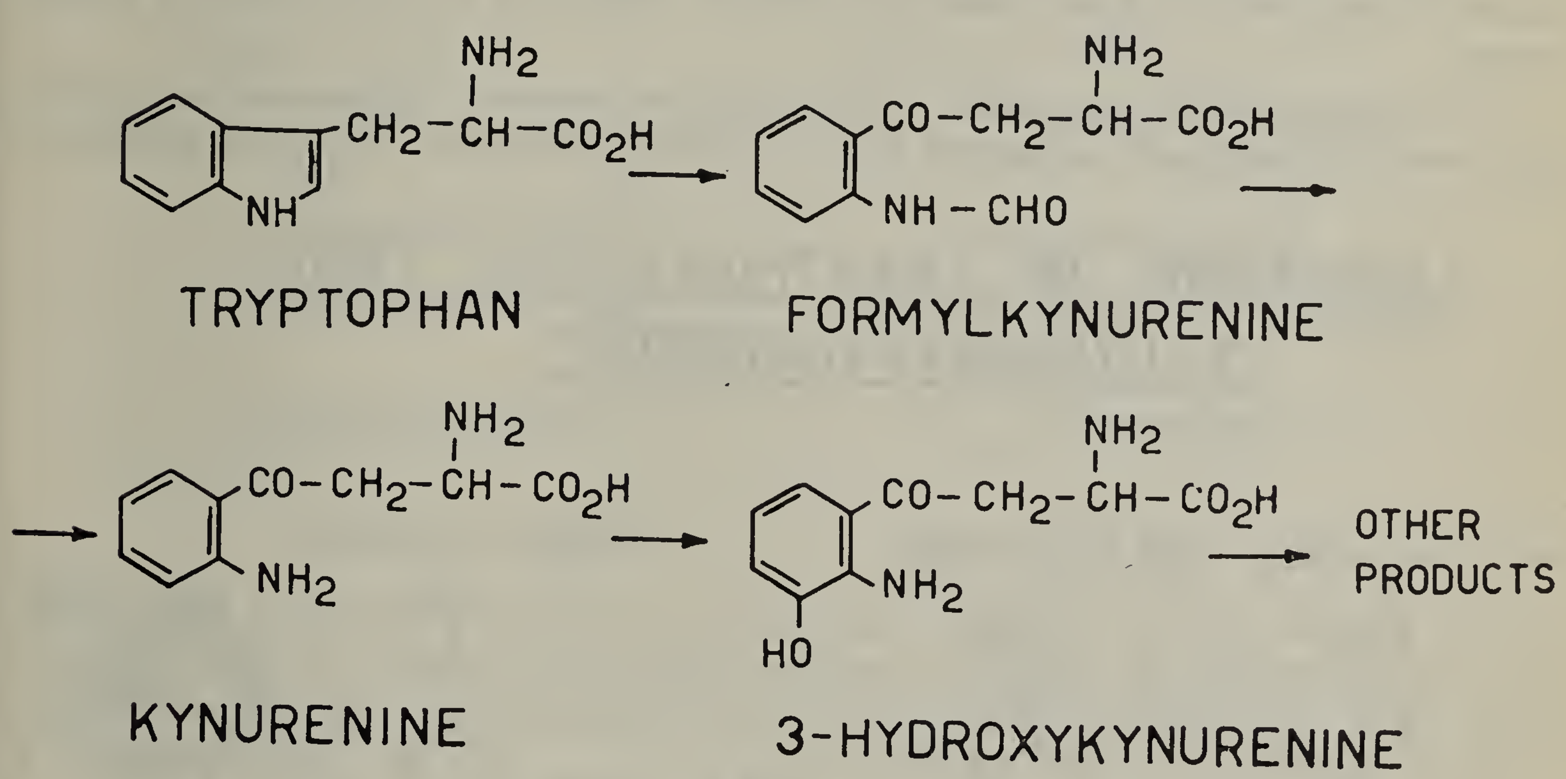
BIOGENESIS OF 4,9-DIHYDROXYPERYLENE-3,10-QUINONE IN DALDINIA CONCENTRICA



ENDOGENOUS PIGMENTS FORMED BY METABOLISM OF AMINO-ACIDS

Ommochromes. It is now well known that tryptophan is metabolised by moulds, bacteria and higher animals, by the following route.

METABOLISM OF TRYPTOPHAN



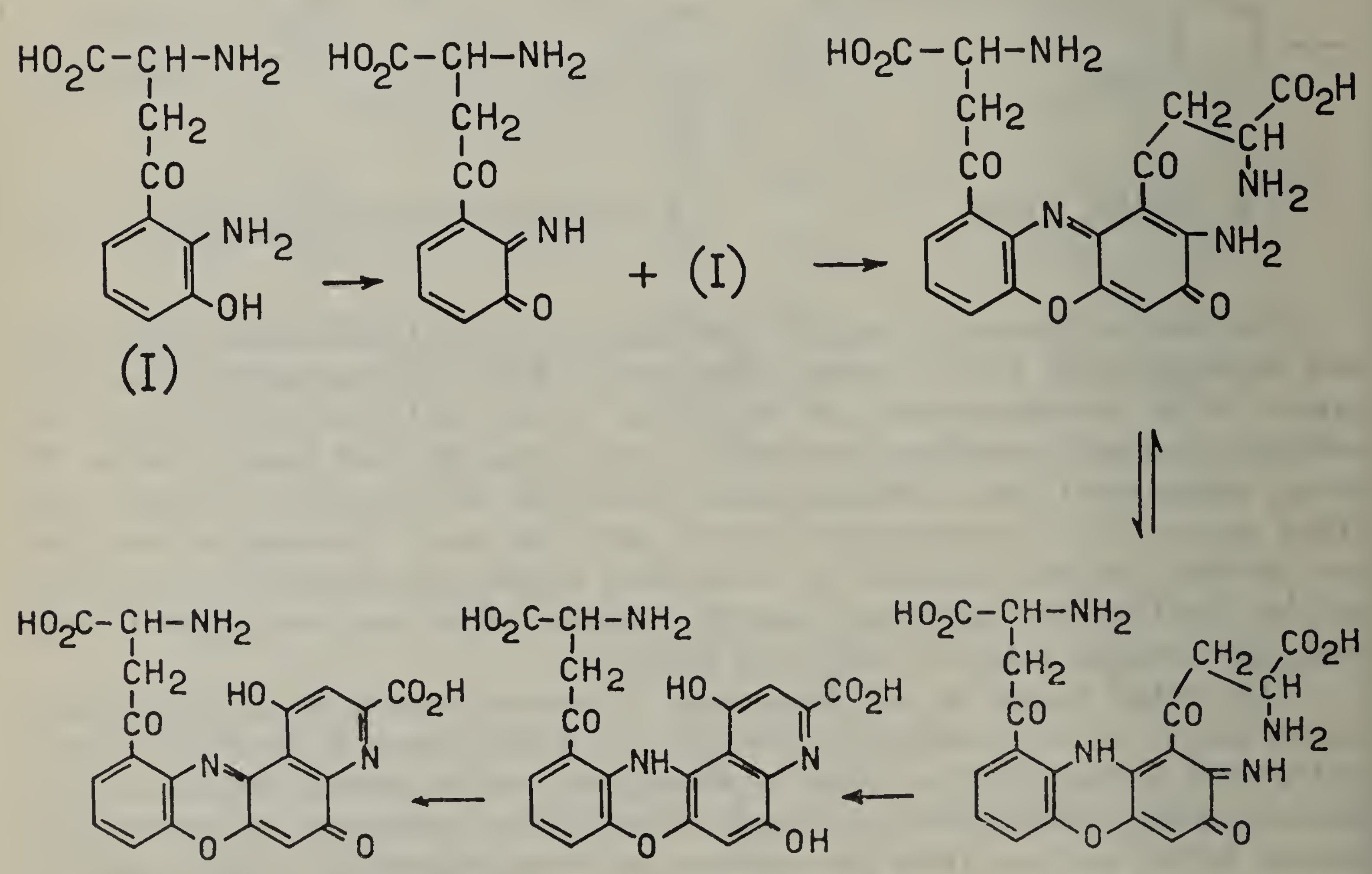
The « other products » include xanthurenic acid, 3-hydroxyanthranilic acid and nicotinic acid, but in many arthropods 3-hydroxykynurenine is the precursor of the ommochromes, an important group of brown, yellow and red colouring matters occurring especially as eye pigments, but found also in the wings, integument, and metamorphosal secretions of butterflies, crickets, and other species. The ommochromes which are extremely intractable materials, are divided into two classes; the ommatins which are acidic, low molecular weight, alkali-labile compounds, and the ommins which are compounds of high molecular weight, relatively stable to alkali.

The initial stages in the formation of ommochromes from tryptophan, shown above, were established by the classical genetic work of Beadle, Ephrussi and Tatum (30) on the eye colour of *Drosophila melanogaster* mutants and the chemical work of Butenandt on the structure of the chromogen, 3-hydroxykynurenine. In the last ten years the outstanding chemical work of Butenandt (31) and his school in Munich, has elucidated the subsequent reactions of 3-hydroxykynurenine and a total synthesis of two of the ommatins has been achieved. The red moulting fluid of *Vanessa urticae* was found to be the most convenient material for the isolation of pure pigments, and xanthommatin, rhodommatin and

ommatin D were obtained from this source. All three were shown to be ommochromes by injecting *Vanessa* larvae with ^{14}C-labelled tryptophan and kynurenine; the ommatins subsequently separated from the metamorphosal secretion were radioactive. Crystalline xanthommatin has also been isolated from the eyes of the blowfly *Calliphora erythrocephala*, and here again injection of labelled tryptophan into the pupae led to the formation of radioactive pigment in the newly hatched flies. Butenandt's scheme for the *in vitro* formation of xanthommatins by oxidative condensation of two molecules of 3-hydroxykynurenine is shown below and it is likely that the enzymic oxidation *in vivo* follows a similar course.

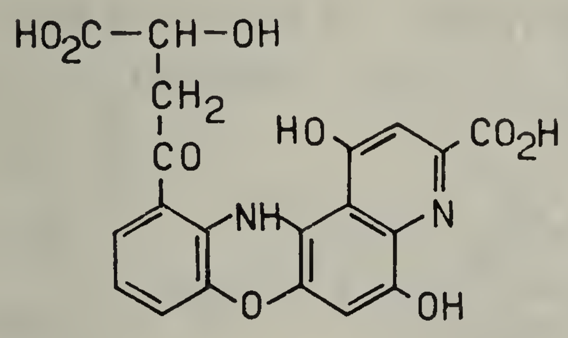
The ommatins as thus a new type of pigment having a phenoxazone nucleus. The same chromophore appeared later in the actinomycins (32) (red antibiotics

FORMATION OF XANTHOMMATIN FROM 3-HYDROXYKYNURENINE

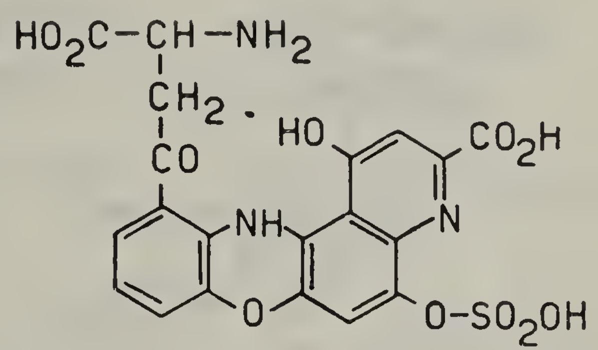


XANTHOMMATIN (yellow - brown) DIHYDROXANTHOMMATIN (deep red)

produced by Actinomycetes) and in the fungal pigment cinnabarin (polystictin) (33). Characteristically these cmpounds are reduced, not to colourless leuco forms, but to deep red dihydro-ommatins. Both states of oxidation occur in Nature sometimes in different regions of the same species or at different stages of development, e.g., xanthommatin is responsible for the dark brown markings on the larvae of *Cerura vinula* which change dramatically to bright red shortly before pupation owing to reduction of the pigment to dihydroxanthommatin.



RHODOMMATIN

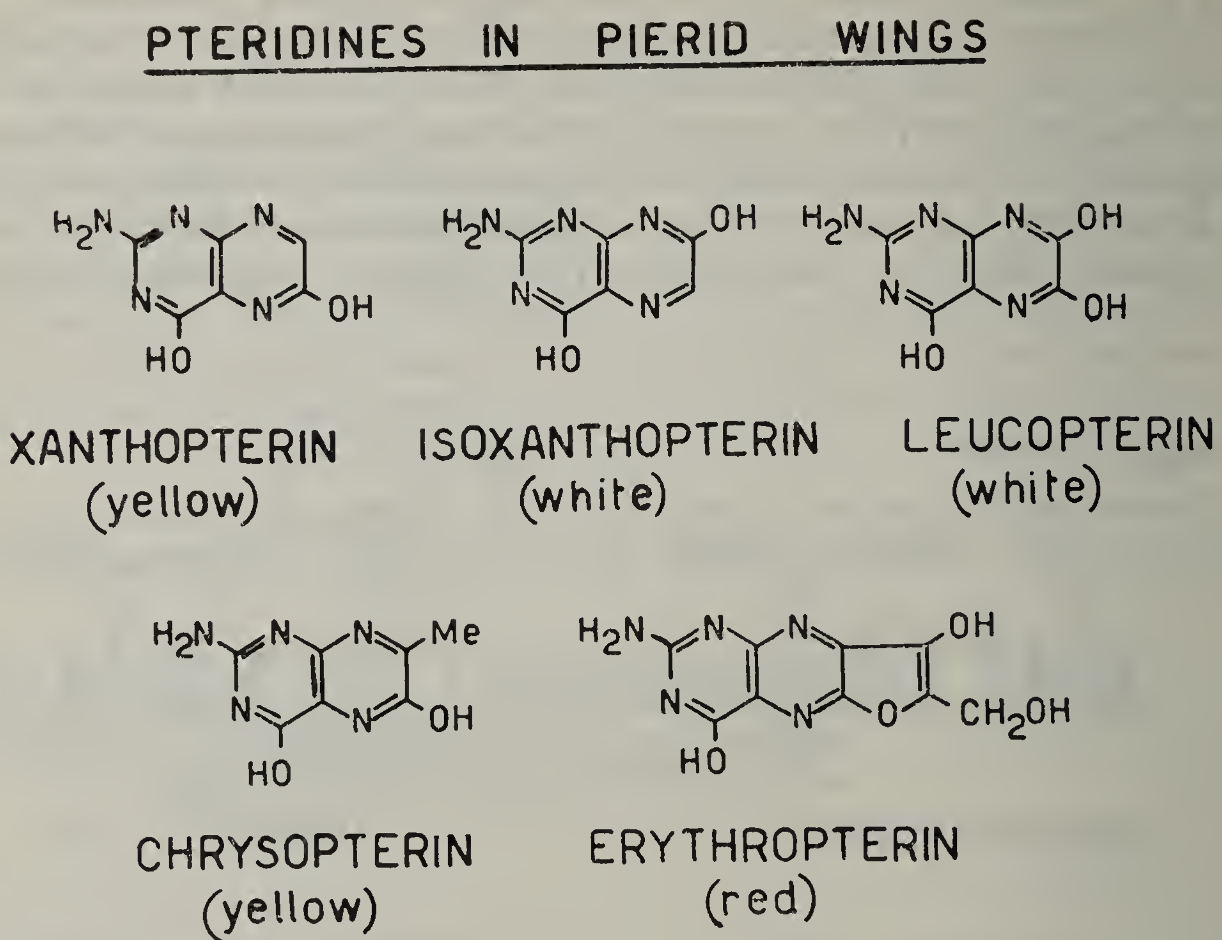


OMMATIN D

Bückmann (34) has concluded recently that both the colour change and the pupal moult are caused by the same hormone, ecdyson, in different concentrations. Rhodommatin and ommatin D however, occur naturally in a red reduced form.

The isolation and purification of the ommins is a very difficult task on account of their particularly intractable nature. It is a measure of the labour involved that Butenandt and his co-workers (35) obtained less than 1g. of pigment from the heads of 180,000 silkworms. Recent work (36) has revealed that the heads of *Bombyx mori* contain several (at least five) ommins, the major component, ommin A, being a black-violet compound, $C_{30}H_{27}N_5O_{10}S.H_2O$, which breaks down on acid hydrolysis yielding 3-hydroxykynurenine and an ommatin-like pigment $C_{20}H_{15}N_3O_7S$.

Pteridines. These nitrogenous compounds have long been known as butterfly wing pigments but their investigation is not an easy matter. Originally discovered by Gowland Hopkins in 1891, fifty years were to elapse before Purrmann established the constitution of leucopterin in 1940. In the next decade, the structures of several others were determined, the growth stimulating properties of folic acid were discovered, and it became clear that pteridines occurred widely in the animal kingdom, and in lower plants. However, it is worth recalling today, that the pteridine ring system was originally discovered by workers interested in insect colouring matters. The better known wing pigments are shown below.



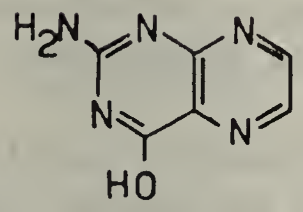
Pterins are found also in the pupae of *Lepidoptera,* in the bands of wasps, together with ommochromes in the eyes of many *Diptera,* and elsewhere. It will be noted that all the naturally occurring pteridines have an amino group at position 2, and a hydroxyl group at position 4, a structural feature which produces a high degree of insolubility and adds much to the difficulty of handling these materials.

Reference has already been made to genetic studies on the eye pigments of *Drosophila melanogaster.* The genetic interrelationship of the various eye colour mutants are well established but a fuller understanding of gene-linked pigment formation requires a complete chemical analysis of the process. In this respect work on the ommochromes has outstripped the pteridine investigations; the latter however are complicated by the co-occurrence of an embarrassing number of pigments, some of which are unstable. This was first revealed by Hadorn and co-workers who showed that extracts of different mutants of *D. melanogaster* (37) and *Ephestia kühniella* (38) gave a characteristic pattern of fluorescent spots when developed on two dimensional chromatograms. Seven fluorescent materials were detected in the wild type of *Drosophila* and a close chemical relationship was at once evident. This has been confirmed by the structural determinations of Forrest (39) in the U. S. and Viscontini (40) in Zürich during the last few years, and the following pteridines have now been isolated from *D. melanogaster.*

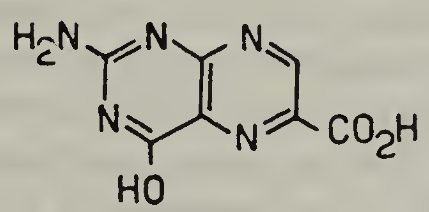
PTERIDINES IN DROSOPHILA MELANOGASTER

XANTHOPTERIN
(greenish-blue fl.)

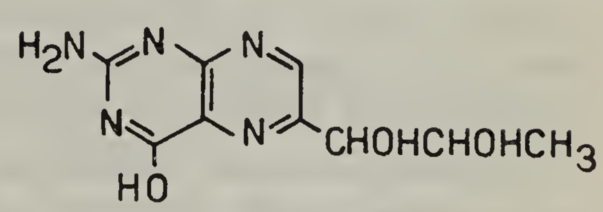
ISOXANTHOPTERIN
(violet-blue fl.)



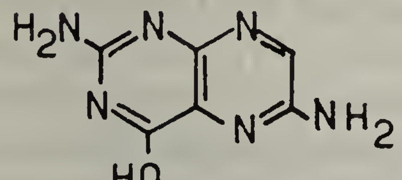
2-AMINO-4-
HYDROXYPTERIN
(sky-blue fl.)



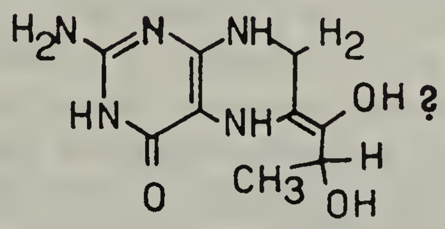
2-AMINO-4-HYDROXY-
PTERIN-6-CARBOXYLIC
ACID



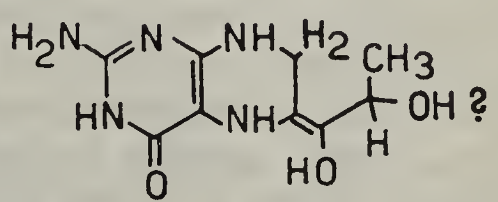
BIOPTERIN
(blue fl.)



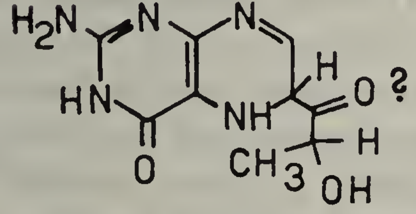
2,6-DIAMINO-4-
HYDROXYPTERIN
(yellow, greenish-
yellow fl.)



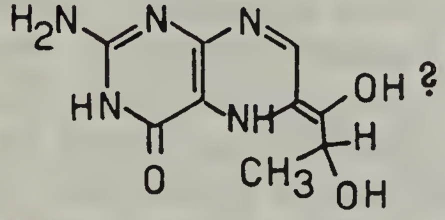
SEPIAPTERIN
(yellow, yellow fl.)



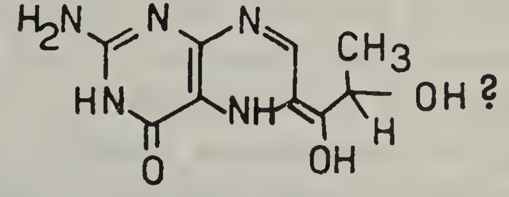
ISOSEPIAPTERIN
(yellow, yellow fl.)



NEODROSOPTERIN
(red, orange fl.)



DROSOPTERIN
(red, orange fl.)

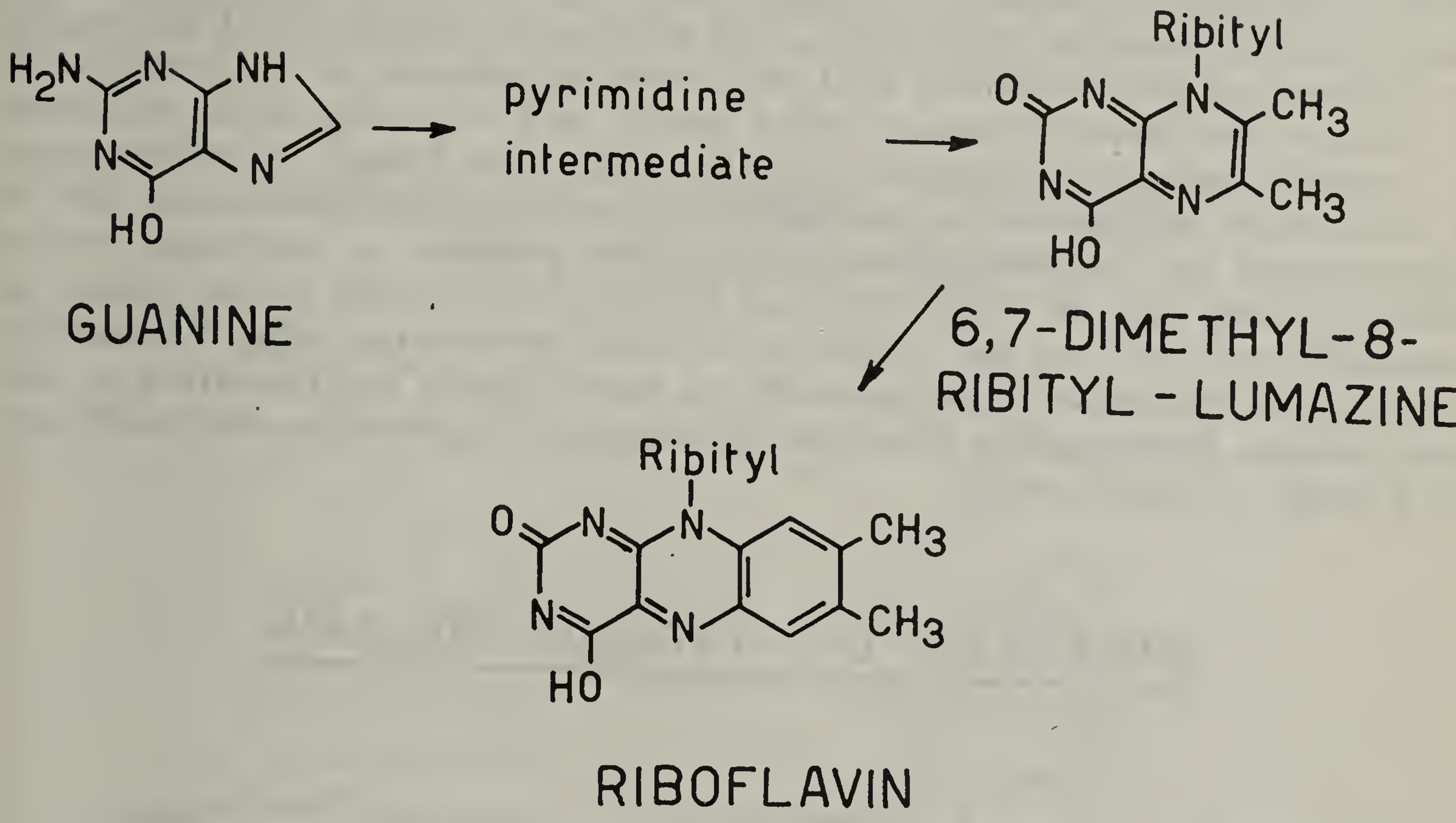


ISODROSOPTERIN
(red, orange fl.)

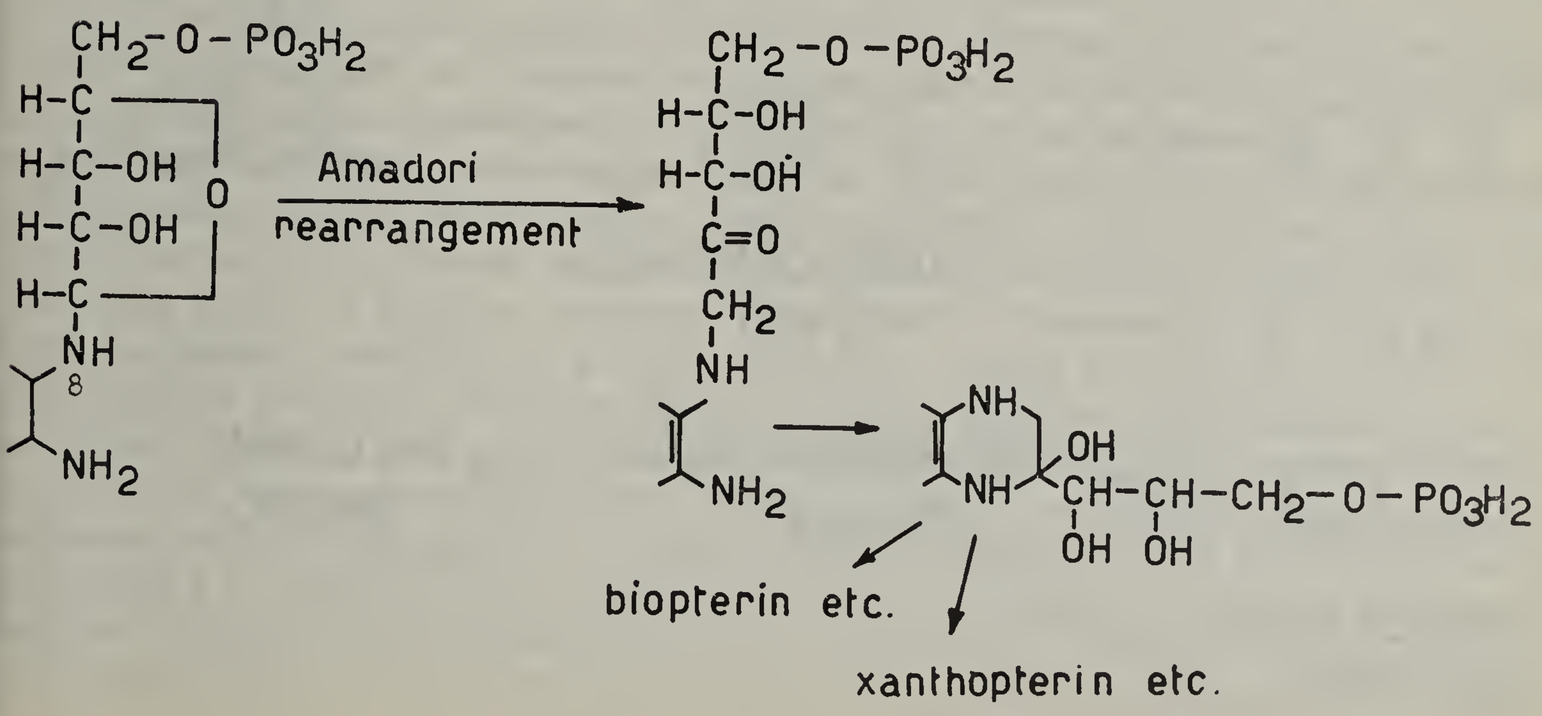
The side chain at C_6 in biopterin, sepiapterins and drosopterins exists in several stereochemical configurations and is photo-labile, these compounds being readily degraded in the presence of light to 2-amino-4-hydroxypterin-6-carboxylic acid. It is known that 2-amino-4-hydroxypterin is the direct precursor of isoxanthopterin but 2,6-diamino-4-hydroxypterin (39) may be an artefact formed by the action of ammonia during the isolation procedure. Only small amounts of the yellow sepiapterins are present in wild type *D. melanogaster* flies but considerable amounts accumulate in the eyes of the *sepia* (*se*) mutant which does not produce red pigments. It appears from this that the yellow compounds are precursors of the red ones. Viscontini (40) formulates the yellow sepiapterins as tetrahydropteridines and the red drosopterins as dihydropteridines. It is suggested that the sepiapterins and corresponding drosopterins are *cis-trans* enol isomers, neodrosopterin having the keto form.

As may be seen, studies on the structure and isolation of natural pteridines are making steady progress and these compounds now form the largest group of insect pigments. By contrast little is known of the biogenesis of the pteridine ring system. It has generally been supposed that they are derived from purines, which frequently accompany the pteridines, and are themselves formed by a lengthy biosynthesis involving glycine, glutamine, aspartic acid, formate and carbon dioxide. Conversion of a purine into a pteridine can be achieved *in vitro* but there is little evidence in favour of the *in vivo* process with the notable exception of riboflavin biosynthesis. The formation of riboflavin, which might be regarded as a benzpteridine, from purines (e.g., guanine) by *Eremothecium ashbyii, Ashbya gossypii* and other moulds is established (41), and moreover the pteridine, 6,7-dimethyl-8-ribityl-lumazine is an intermediate in this process. However these moulds are rather exceptional in producing large amounts of riboflavin and it is by no means established that this is the normal pathway for pteridine biogenesis. Some insight into the course of events in insect metabolism has been obtained from feeding experiments with labelled compounds. In this way Waygand (42) and coworkers have shown that the biogenesis of leucopterin in *Pieris brassicae* is similar to purine biogenesis insofar as glycine provides the two central carbon atoms and C_2 is derived from formate. They also found that D-glucose(1-^{14}C and 2-^{14}C) and D-ribose(1-^{14}C) provided C_6 and C_7. Somewhat similar results have been obtained by Brenner-Holzach and Leuthardt (43) using *Drosophila melanogaster* larvae, who found that carbon atoms of glucose (or an immediate metabolite thereof) were specifically incorporated into drosopterin and isoxanthopterin by the *wild type*, and into sepiapterin and isoxanthopterin by the *sepia* mutant. These results are in harmony with the hypothesis that the pyrazine ring arises by cyclisation of an N-ribityl precursor, leading to the formation of a pteridine bearing a three carbon side chain at C_6. Pteridines of the biopterin type could then arise by modification of the side chain; alternatively oxidative fission could produce xanthopterin and related pigments (42 a). Much remains to be discovered but the general picture of pteridine biogenesis is becoming clearer.

BIOSYNTHESIS OF RIBOFLAVIN IN EREMOTHECIUM ASHBYII

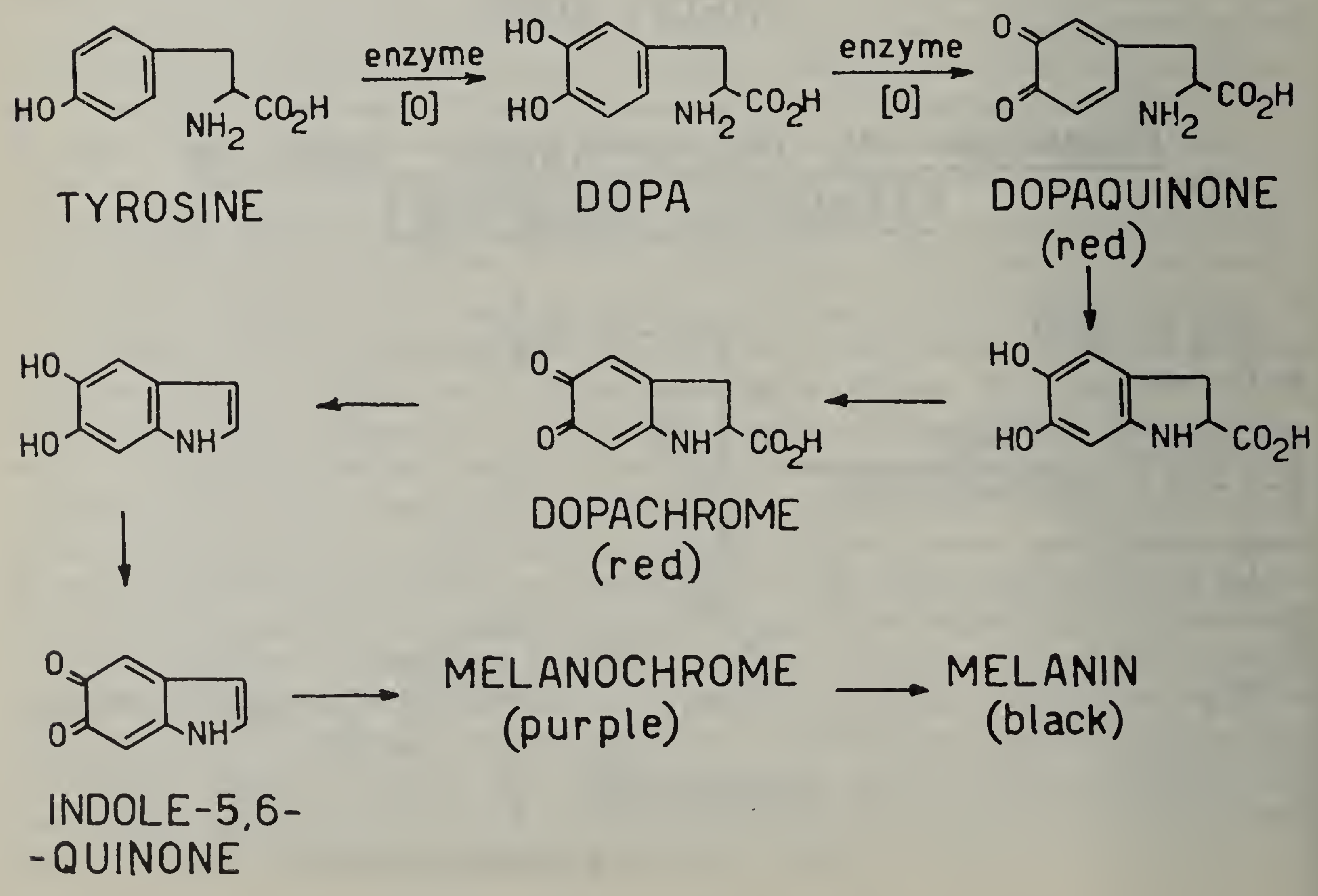


FORMATION OF THE PYRAZINE RING IN PTERIDINE BIOGENESIS



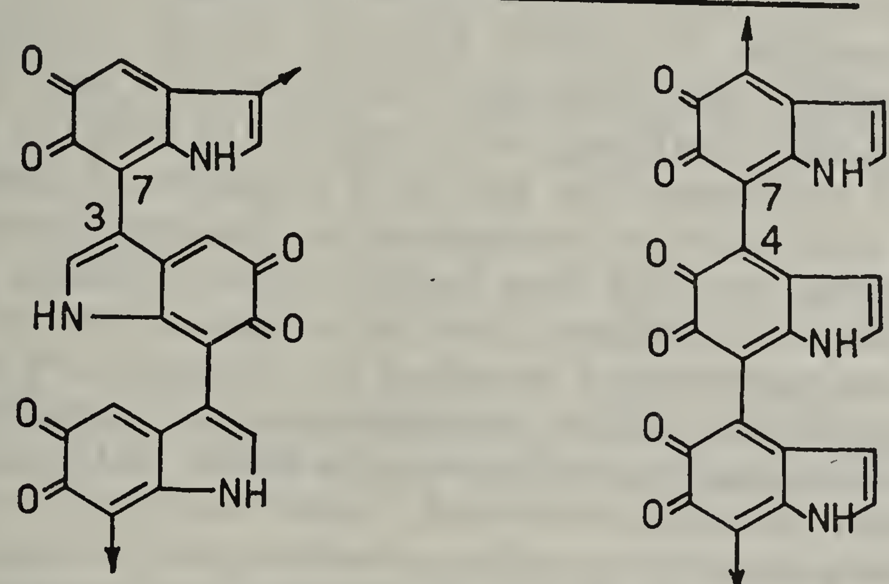
MELANINS (44). These are defined as black or brown, polymeric pigments formed by tyrosinase oxidation of tyrosine or such closely related compounds as β-3,4-dihydroxyphenylalanine (dopa), tyramine and 3-hydroxytyramine[β-(3,4-dihydroxyphenyl)ethylamine]. Melanin formation is frequently observed when insect blood, which contains tyrosine, is exposed to air, but generally the pigment is incorporated in the cuticle. The position is complicated however, by the presence of protocatechuic acid and other polyphenols in the cuticle, and by the fact that insect tyrosinase is not specific and will çatalyse the oxidation of a range of mono- and di-hydric phenols. It has been difficult, in consequence, to disentangle the process of melanisation from cuticular darkening due to sclerotisation, but the independence of the two processes in now clearly established by the work of Malek (45) and Dennel (46), and the recent studies of Fuzeau-Braesch (47) on the cuticle of *Gryllus bimaculatus* using ^{14}C-labelled tyrosine. As tyrosinase occurs generally in insect tissues the formation of melanic patterns indicates that either the chromogen is localised, or that inhibitors are present in certain areas.

BIOGENESIS OF TYROSINE-MELANIN



Owing to its polymeric nature, its lack of homogeneity, close association with protein, and almost complete insolubility in organic solvents, melanins cannot be handled like other pigments of low molecular weight. Moreover insect melanins seldom occur in granular form (as in most animals) which aggravates the difficulty of isolating the pigment, and in fact no direct attempt appears to have been made to examine insect melanin by orthodox methods.

SUGGESTED "BACKBONE" STRUCTURES FOR TYROSINE-MELANIN



(Harley-Mason) (H.S. Mason)

Our knowledge of the structure of melanin is derived mainly from the classical work of Raper (48) on the tyrosine-tyrosinase reaction *in vitro*, the much later model experiments of Harley-Mason (49) and the recent degradative studies of Nicolaus on mammalian melanins and *Sepia*-melanin. The initial stages in the formation of tyrosine-melanin, are set out on page 38.

It is generally accepted that melanin is ultimately formed by oxidative condensation of the very reactive indole-5,6-quinone. On the basis of model experiments Harley-Mason suggests that condensation occurs mainly at the 3-7 positions to give a polymer having a « backbone » structure of the form shown, with occasional crosslinks at position 2-4 or 2-7 giving a highly irregular three-dimensional polymer. However the degradative evidence of Nicolaus, obtained both from natural and « synthetic » melanins, indicates that position 3 is not involved to any appreciable extent in the oxidative condensation of indole-5,6-quinone and accordingly H. S. Mason (50) has proposed that tyrosine-melanin is mainly a 4-7 linked polymer. There is strong presumptive evidence that mammalian melanins and *Sepia*-melanin are structurally related to the synthetic melanins studied *in vitro*, with the addition of protein firmly bound to the « back-

bone » structure. As insect melanins are also formed in a system containing tyrosine and tyrosinase it is not unreasonable to suppose that they are structurally similar.

RELATIONSHIP BETWEEN OMMOCHROMES, PTERIDINES AND MELANINS

These pigments of diverse chemical structure, frequently occur together, e.g., ommochromes and pteridines are found in the eyes of *Drosophila* and *Ephestia*, and pteridines and melanins in the wings of many butterflies, and some sort of biochemical relationship might be expected. There is some evidence (51) that pteridines can effect the course of melanogenesis and this may contribute to the formation of melanic patterns, already mentioned, but the mechanism of this interaction is obscure. In *Drosophila* and *Ephestia* many mutations are known which influence the formation of both the brown ommochromes and the red pteridines. The red- eyed *a* mutant of *Ephestia* accumulates pteridines but is deficient in ommochromes. Injecting kynurenine into *a* pupae however restores the *wild type* pattern, ommochromes become more abundant and the pteridines decline. Thus the *a* gene affects both the tryptophan ——> kynurenine reaction and somehow the formation of pteridines. In *wa* mutants the biosynthesis of both ommochromes and pteridines is blocked. From a close study of various eye-colour mutants Kühn (52) concludes that the last stages of pigment formation (of both types) take place within protein granules and that the pigments (or their precursors) are in competition at the reaction sites. Another hypothesis (53) which has been put forward is that one of the intermediates in the pteridine biosynthesis my function as a cofactor for one of the oxidative steps in the formation of ommochromes. The enzymes responsible for the latter have not yet been identified but Butenandt (54) has shown that tyrosinase will catalyse the oxidation of 3-hydroxykynurenine to xanthommatin in the presence of small amounts of dopa or catechol. It is not clear whether the latter serve to activate (i.e. reduce) the enzyme or whether, as Butenandt holds, an *o*-quinone is formed which in turn dehydrogenates the hydroxykynurenine. If this system functions *in vivo* we should expect to find some relationship between ommochrome and melanin formation. The « red melanin » obtained from the *rb* mutant of *Bombyx mori* by Inagami (55) appears to be a mixture of both.

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SUMMARY

The purpose of this lecture is to review the present state of knowledge concerning the origin and biogenesis of the principal groups of insect pigments, and to draw attention to problems requiring further investigation.

Despite much work in the last twenty years our knowledge of these colouring matters is still comparatively limited. Less than thirty pigments have been identified with certainty. Pteridines and ommochromes are responsible for most of the red, orange, yellow and brown, pigmentation, whilst leucopterin and isoxanthopterin provide white, and the melanins, black. Pigmentary green is usually a combination of yellow (carotenoid) and blue («bile pigment») chromoproteins. The pteridines, ommochromes and melanins are product of nitrogen metabolism and it has yet to be established if any of the non-nitrogenous colouring matters are true products of insect biosynthesis. The carotenoids and flavones (?) are almost certainly exogenous, and it is possible that the anthraquinone and aphin pigments, elaborated by sap-sucking insects, are synthesised from sugars by symbiotic micro-organisms.

Inspection of formulae suggests that the polycyclic quinones are biosynthesised from acetate units, a process known (to date) only in the plant kingdom. The occurrence of glycosides, e. g., carminic acid, protoaphins, is also unusual amongst animal pigments.

The biogenesis of the nitrogenous colouring matters is more securely based. The formation of melanin from tyrosine is understood, at least in the initial stages, and the derivation of the ommochromes from tryptophan is established, but the origin of the pteridines, the most numerous group, is not yet clear.

RIASSUNTO

Pigmenti degli Insetti.

Lo scopo di questa conferenza è di rivedere il presente stato di conoscenza riguardante l'origine e la biogenesi dei principali gruppi dei pigmenti degli Insetti e di attrarre l'attenzione verso problemi che richiedono ulteriori indagini.

Nonostante il molto lavoro compiutosi negli ultimi venti anni la nostra conoscenza di queste sostanze coloranti è tuttora relativamente limitata. Meno di trenta pigmenti sono stati identificati con sicurezza. Pteridine e ommocromi sono responsabili per la maggior parte delle colorazioni rosse, arancio, gialle e marrone, mentre la leucopterina e la isoxantopterina sono responsabili della colorazione bianca e la melanina di quella nera. La pigmentazione verde è di solito una combinazione di cromoproteine gialle (carotenoide) e blu (pigmento della bile). Le pteridine, ommocromi e melanine sono prodotti di metabolismo azotato e si deve ancora stabilire se ogni materia colorante non azotata sia un vero prodotto della biosintesi degli Insetti. Carotenoidi e flavoni (?) sono quasi sicuramente esogeni ed è possibile che i pigmenti antrachinone e afini elaborati da Insetti succhiatori di linfa, siano sintetizzati da microrganismi simbiotici a partire dagli zuccheri.

Un esame di formule suggerisce che chinoni policiclici possano essere biosintetizzati da unità acetiche con un processo conosciuto (finora) soltanto nel regno delle piante. Il trovare glicosidi (per es. acido carminico, protoafini) è anche insolito fra i pigmenti animali.

La biogenesi delle sostanze coloranti azotate ha basi ancor più salde. La formazione di melanina dalla tirosina è intuita almeno negli stadi iniziali, ed è stabilita la derivazione degli ommocromi dal triptofano. D'altra parte l'origine delle pteridine, il gruppo più numeroso, non è chiara.

BEARD R. L. (*)

THE NATURE OF CERTAIN ARTHROPOD VENOMS AND THEIR EFFECTS ON INSECT PHYSIOLOGY

Chemicals having remarkable properties are secreted by the salivary and poison glands of many spiders and insects. In some respects the nature of these chemicals is no better known than when Hase in 1924 and Nielsen in 1935 reported that these venoms from parasitic and predatory insects have not been widely studied, chiefly because of the difficulties in obtaining experimental material in quantity. To be sure, we are coming to understand the chemistry of arthropod venoms poisonous to man. The venoms poisonous to insects are a different matter.

For purposes of this symposium it would be most appropriate to characterize in detail the chemistry of these venoms and draw comparisons of their constituents. This I cannot do. In some cases the physiologic effects of these substances are not due to their chemistry alone. On the other hand, the physiologic effects may suggest the chemical nature of the venom. So I would like to call attention to the nature of these secretions in terms of their physiological properties.

As a group, these agents toxic to insects include what might be called the most general insecticides, the most specific insecticides, the most potent insecticides, and the fastest acting insecticides.

The toxins having most general actions are those secreted by spiders and the predatory reduviid and pentatomid bugs, neuropterous and tabanid larvae. In contrast, the venoms of some of the braconid or sphecid wasps are so specific as to affect single or few species of host.

The fastest insect killing agent I know is the bite of a tabanid larvae. An innocent looking nip from this predatory insect can instantaneously stop all evidence of life in its prey. Even electrophysiological methods detect no sign of life.

The most potent venoms I know are those of braconid wasps. The venom of the black-widow spider (*Latrodectus mactans*) is also highly potent against insects, but even this is not in the same range as the braconid venom.

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A comparison of venoms and their effects on insects does not lead to simple generalizations. Nevertheless the properties of host specificity, potency, speed of action, and the effect on the host can be found to result from and to be affected by the chemical nature of the toxin, its volume and concentration, and the manner in which it is injected.

The chemical nature of venoms is of prime concern to all four of the properties just mentioned. Satisfactory chemical analysis has been made of some venoms poisonous to man (see Kaiser and Michl, 1958). With respect to those poisonous to insects we can do little more than generalize as to the nature of the chemical involved. The non-specific venoms are chiefly corrosive in their action. Their cytolytic action must result from proteolytic and lipolytic enzymes. Edwards (1960) has verified this in reduviid bugs, and other work on spider venoms emphasizes the proteolytic components. Lecithinase is a possible constituent in some venoms, but it is probably not widespread. The effectiveness of some venoms is doubtless increased by hyaluronidase, but its range of occurrence is not known. Such enzymes adequately account for the immediate lethal action of these general toxins and for the prompt degenerative changes that occur in the victims.

Contrasting sharply with such general toxins having general effects, there are many parasitic hymenoptera which immobilize prey momentarily or for very short periods of time. In these the lytic enzymes must be largely lacking *Phytodietis*, a parasite of the spruce budworm is one example. The sting of this wasp induces in its host an immediate paralysis which passes off within ten minutes. Such a temporary action makes study difficult, but it suggests a surface acting narcotizing agent of high potency, but unstable in the internal milieu of the host.

In a third type of action, the venom induces an irreversible paralysis, with death resulting. It is not likely that the chemical involved is merely a more stable or more potent narcotic than the type just mentioned. It tends to be specific not only as to host species affected, but as to tissues that are inactivated. Braconid, sphecid, thynnid, and pompillid wasps provide examples of insects producing this type of venom. For the most part the action of the chemical is to inactivate excitation of the somatic muscles. Visceral muscles and nervous tissues are generally unaffected, suffering only from secondary degenerative changes. It may be, however, that venoms of some sphecid wasps contain proteolytic enzymes in addition to a paralyzant and thus induce primary cytotysis along with paralysis. The most attractive hypothesis of the paralizing mechanism is that the venom molecularly substitutes for a critical muscle substrate sensitive to the mediator of nerve impulses. Certainly a protein, protein-like substance, or a substance attached to a protein is involved. Evidence for this is found in the venom of *Microbracon hebetor*. It is destroyed by heat, is non-dializable, and can be precipitated with half saturated ammonium sulfate. It can be adsorbed on calcium phosphate gel and eluted with

alkaline buffer. It is water soluble, but is relatively stable when dry. Preliminary chromatographic and electrophoretic techniques have not been very helpful in analyzing components, but they have not been adequately tried. The synthetic approach of following tracers in suspected precursors of the venom is an intriguing possibility. So far it has only been shown that phenylalanine marked with C^{14} is not incorporated in the venom.

The potencies of these venoms differ widely. The *Microbracon* venom is the most potent I know. With some assumptions, it is estimated that one part in 200,000,000 parts of host blood is adequate to paralyze *Galleria* larvae (Beard, 1952). In contrast, relatively large volumes are required of *Sphecius* venom to induce a similar type of paralysis in the cicada. In this case a large volume compensates for an apparent low concentration of the active chemical.

Although the speed of action must involve the nature of the chemical and the volume injected, the mode of injection is important. The remarkable speed of action when a tabanid larva bites its victim can be explained on the basis that a sizeable volume of secretion is injected forceably into the body cavity of the victim. On the other hand a type of progressive death in a larval victim of the ant lion is probably due to a slow diffusion of the secretion oozing from the mandibular canals. These two types of action rates can be demonstrated by forceful as compared with diffusive injection of nicotine. The two methods can induce remarkably different effects even with the same quantity of poison.

Let us consider further the *Microbracon* venom. As reared in the laboratory, the sole food of the wasp is the blood of the host insect. This means that the wasp may be considered a biochemical device for converting host blood into a host poison. In other words, the host provides the chemical raw materials for the destruction of its own relatives through the parasite carrier. Viewed in this way, the evolution of species-specific parasites seems easier to understand. This raises the question, though, as to the relative potency of venoms in wasps reared on different hosts. Assay of *Microbracon* venoms from wasps reared on *Galleria* larvae and wasps reared on *Anagasta* larvae indicate similar potencies when tested on *Galleria*. Hence these two bloods are equally adequate for the synthesis of the venom.

Curiously, some venom is present in wasps at all stages of the life cycle, as seen in the following table:

Extracts of 1 mg of:						
Microbracon	EGGS	can	paralyze	250	*Galleria*	larvae
»	LARVAE	»	»	10	»	»
»	PUPAE	»	»	±	»	»
»	MALE ADULTS	»	»	±	»	»
»	FEMALE ADULTS	»	»	250,000	»	»

A fair amount is present in the eggs, less in the larvae. In the pupae enough is present to sometimes paralyze *Galleria* larvae, but there is too little to evaluate. The same is true of male wasps, but female wasps are, of course, richly supplied. The presence in the egg and subsequent stages in decreasing amounts suggests that the eggs are contaminated with the venom, which gradually dissipates with continued growth and development. The synthesis and elaboration of venom in the female wasp after emergence constitutes a biochemical maturation. A less likely alternative is that the presence of venom in other stages indicates the venom to be a normal metabolite such as a sex hormone and the female poison apparatus constitutes a contentrating and accumulating mechanism.

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SUMMARY

Amazing drugs active in insects are secreted by salivary and venom glands in predatory insects and spiders. Two types of action predominate: 1) the secretion is outright insecticidal, killing promptly, probably because of strong protease activity; 2) the secretion inactivates the somatic but not the visceral neuromuscular system to cause temporary or permanent paralysis. Producers of these poisons follow no taxonomic pattern. The potency of these poisons varies widely. Their rates of action involve the volume, potency, and nature of the chemical injected into the prey. Some of these aspects are compared for different venoms, particularly the venom of *Microbracon hebetor.*

RIASSUNTO

La natura di alcuni veleni di Artropodi e i loro effetti sulla fisiologia dell'insetto.

Sorprendenti sostanze attive sugli insetti sono secrete da glandole velenose e salivari di Insetti predatori e Ragni. Predominano due tipi di azione: 1) la secrezione è senz'altro insetticida, la morte subitanea, soprattutto a causa della forte attività proteasica; 2) la secrezione blocca il sistema nervoso somatico ma non quello neuromuscolare viscerale causando paralisi temporanea o permanente. La presenza di questi veleni non è in relazione alla sistematica zoologica. La loro potenza varia moltissimo. I loro gradi di azione sono in relazione al volume, alla potenza e alla natura del fattore chimico iniettato nella preda. Alcuni di questi aspetti sono paragonati per i differenti veleni, soprattutto per il veleno di *Microbracon hebetor.*

Blum M. S., Traynham J. G. (*)

THE CHEMISTRY OF THE PENTATOMID SCENT GLAND

In many of the Hemiptera, specialized glands have developed from which highly odoriferous compounds are ejected. Because they eject very odoriferous substances when disturbed, the Pentatomidae have been called stink bugs. This paper described the analyses of some of the scent gland components found in the rice stink bug *Oebalus pugnax* (F).

METHODS AND MATERIALS

The contents of the scent gland were collected by piercing exposed glands with fine capillaries.

Infrared analyses were made on a Perkin-Elmer Model 21 spectrophotometer both from a film and from a carbon tetrachloride solution.

Vapor phase chromatographic analyses were performed on a Perkin-Elmer Model 154 B vapor phase chromatograph by injecting 1-50 μl. samples into the inlet of the instrument. Tris-phenoxyphenyl-*n*-dodecyl silane was used as an adsorbant; the instrument was operated at 160° C. Samples were collected by condensing the components in microtubes immersed in liquid nitrogen as they issued from the outlet of the instrument.

Mass spectrographic analyses were made on a modified Consolidated 21-102 analytic mass spectrometer.

2,4-Dinitrophenylhydrazones were prepared by adding the scent gland fluid to 5 ml. of absolute ethanol to which was added a saturated solution of 2, 4-dinitrophenylhydrazine in 2N HCl. The alcohol soluble derivatives were chromatographed employing the method of Gordon *et. al.* (1951).

RESULTS

The fluid which had been freshly removed from the scent gland cointained a suspension of orange droplets suspended in a clear fluid. Upon settling, the orange droplets formed a lower layer which consisted of about 40 per cent of the total sample.

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The infrared spectrum indicated that the scent gland secretion contained aldehydic carbonyl components as well as an alcohol and an acid. The unsaturation present was indicated as *trans*-species (10.25 μ). Furthermore, the C-H/C = O ratio indicated that a high percentage of a hydrocarbon species was present in the mixture. The C-H absorption was entirely aliphatic and the weak CH_3 absorption compared to the CH_2 indicated low branching.

A system of considerable complexity was indicated by the analysis of the *Oebalus* secretion on the vapor phase chromatograph. Eight components were usually present (Fig. 1) and several times an additional ninth high boiling component was detected. Because of the similar retention times of the seven lower boiling components, it was not possible to fractionate them on the vapor phase chromatograph. These components were collected in a single tube for analysis. Because of its longer retention time, the higher boiling component was easily isolated.

The lower boiling components (compounds 1-7) were orange in color and possessed the grassy odor associated with *Oebalus*. Examination of the infrared spectrum of this fraction demonstrated an intense carbonyl absorption. This carbonyl-rich fraction was treated with 2, 4-dinitrophenylhydrazine and the isolated hydrazones characterized.

A fraction of the 2, 4-dinitrophenylhydrazones was sparingly soluble in ethanol and non-polar solvents. Treatment of a few crystals of this fraction with alcoholic potassium hydroxide produced a blue color which indicated the compound was a dicarbonyl compound (Strain, 1935). The ultra-violet spectrum of this derivative in ethanol revealed a series of maxima in the region 400-600 mμ and a maximum at 448 mμ. These absorption maxima are higher than those exhibited by saturated carbonyl compounds (Schepartz and Daubert, 1950) and indicate that the dicarbonyl compound contained a double bond conjugated with the carbonyls. The compound was partially purified by refluxing in ethanol for 48 hours. The derivative melted at 237-240° C. The identity of this compound is still being investigated.

Two alcohol-soluble carbonyl compounds were isolated. One was a bright orange compound which melted at 116-117° C. The ultra-violet spectrum of a sample of this compound in ethanol exhibited a maximum at 377 mμ characteristic of α,β-unsaturation. The small yield of the compound has made more detailed analyses impossible at the present time.

The other alcohol-soluble 2, 4-dinitrophenylhydrazone melted at 130-131° C. and the empirical analysis corresponded to a heptenal (Calculated for $C_{13}H_{16}N_4O_4$, C, 53.42, H, 5.48, N, 19.18 per cent; found, C, 53.61, H, 5.40, N, 19.30 per cent). A melting point in admixture with an authentic sample of 2-heptenal produced no melting point depression. The infrared spectra of the derivative of 2-heptenal and the *Oebalus* derivative were virtually superimposable.

The high boiling fraction isolated from the vapor phase chromatograph exhibited an infrared spectrum characteristic of a saturated hydrocarbon. This

water-clear liquid boiled at 233° C. and melted at —6.1° C. Elemental analysis established an empirical formula of $C_{13}H_{28}$ (C, 24.91, H, 15.06 per cent). The saturated hydrocarbon *n*-tridecane fitted all the analytical data. The linearity of the hydrocarbon was demonstrated by its ability to form a urea addition compound (Rozenberg and Genekh, 1952). The *Oebalus* hydrocarbon and *n*-tridecane had identical retention times on the vapor phase chromatograph.

The identification of *n*-tridecane and 2-heptenal in the scent gland secretion of *Oebalus* further was confirmed by mass spectrographic analysis. The maximum parent mass found with the bug secretion was 184 which corresponds to the parent mass of tridecane. The fragmentation pattern of the high boiling component isolated from the vapor phase fractionation of the *Oebalus* secretion was identical to that of pure *n*-tridecane. If the two phase mixture collected from the gland was allowed to settle, it was found that the clear upper phase contained 80 per cent *n-tridecane* as indicated by peak intensities in the mass spectrograph. The lower phase contained a parent mass of 112 corresponding to a heptenal and exhibited a fragmentation pattern consistent with the peak intensities found in 2-heptenal.

DISCUSSION

The presence of alcoholic and acid species in the scent gland secretion of *Oebalus* suggests that precursory and oxidized components of the aldehydic species are present. The fact that the aldehydic components are not completely soluble in the hydrocarbon *n*-tridecane raises the interesting question of the metastability of the two phases relative to temperature. Certainly the relatively non-volatile hydrocarbon would serve to slow down the rate of evaporation of the dissolved aldehydic components, and thus serve to prolong their existence in the area of ejection.

In many of the pentatomid genera, the scent gland secretions are quite organoleptically distinct. In addition to *Oebalus*, we have studied the scent gland chemistry of two other pentatomids, *Nezara viridula* (L.) and *Euschistus servus* (Say), whose odoriferous secretions are organoleptically distinct. The secretion of *Nezara* is characteristically a sharp grassy odor, whereas that of *Euschistus* is sweetly grassy. The odor associated with *Oebalus* is between those of *Nezara* and *Euschistus*. Nevertheless, the gross chemistry of the scent glands of these three species is similar. Examination of the fluids removed from the glands of these three species shows the presence of a two phase system: orange droplets which settle to form the lower phase leaving a clear upper phase. Mass spectral fragmentation patterns of the gland secretions are virtually identical in all areas where mass fragments carry significant charges.

A basis for the organoleptic differences may be in the proportion of their minor constituents. Usually the scent gland secretion of *Oebalus* contains eight components detectable on the vapor phase chromatograph. The low boiling components which have the odor associated with this bug are represented by six minor components and one major one (6) which rapresents more than 90 per

cent of this fraction (Fig. 1). On the other hand, the scent gland fluid of both *Nezara* and *Euschistus* contain eight low boiling components of which 7 is the major one (Fig. 1). Component 6 in *Oebalus* and 7 in *Euschistus* and *Nezara* are probably identical since in all three cases retention time is identical on the vapor phase chromatograph. On the other hand, in *Oebalus*, component 3 is the predominant minor component present in the low boiling fraction whereas component 8 is the predominant minor one in *Nezara* and component 5 in *Euschistus*. Furthermore, in *Nezara* components 4 and 6 are present in equal quantity but in *Euschistus* components 6 and 8 are the other predominant minor chemical species of relatively equal concentration. It is very unlikely that these minor components are detectable on the mass spectrograph; consequently spectrographic patterns will only reflect the presence of the major components which appear to be identical in the three species. This conclusion is borne out by the facts *n*-tridecane also has been isolated from *Euschistus* and *Nezara* and soluble and insoluble carbonyl compounds are present in both these pentatomids which appear to be identical to those in *Oebalus*.

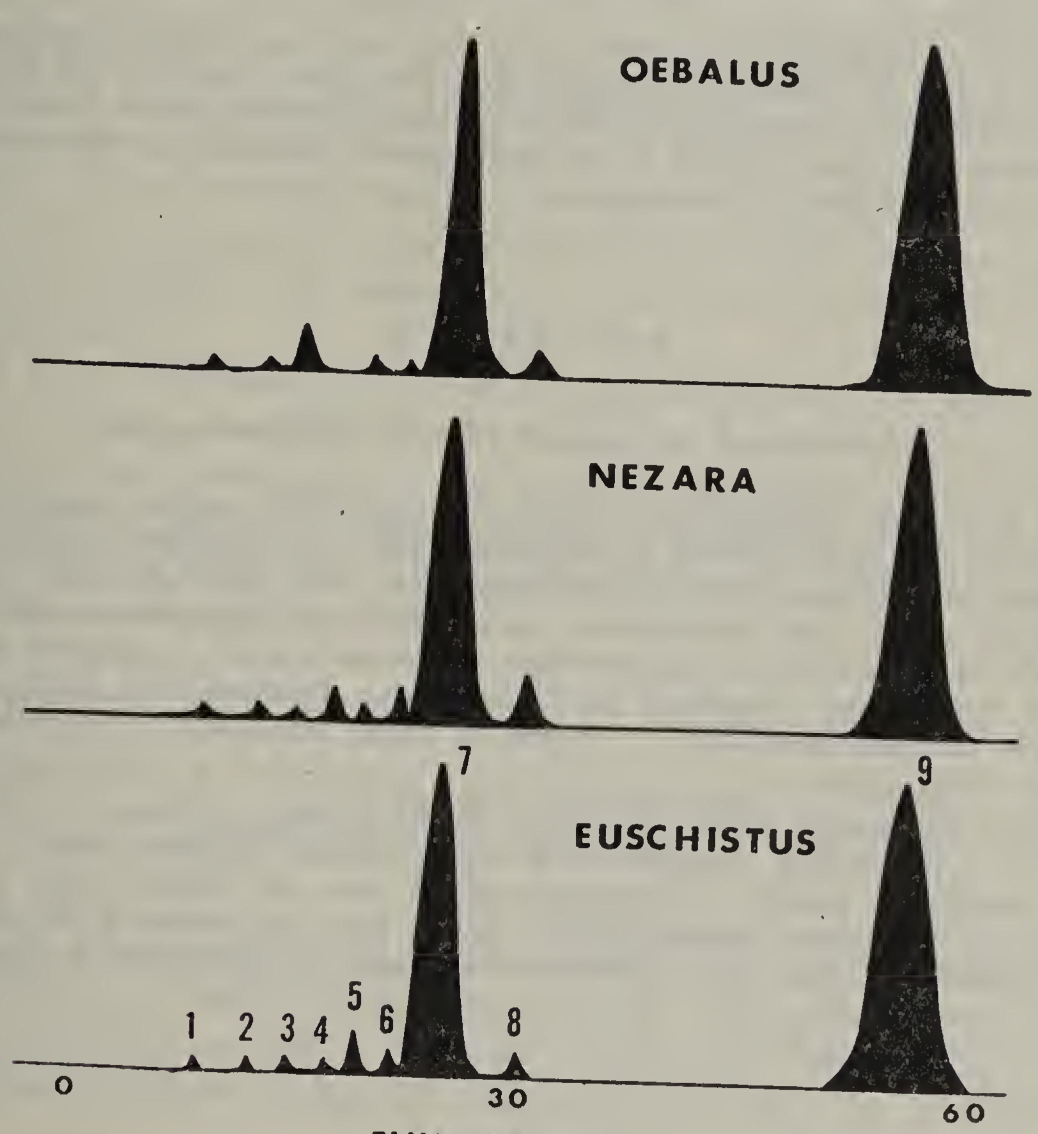

Figure 1.
Vapor phase chromatographic analyses of the scent gland secretions of *Oebalus pugnax* (F), *Nezara viridula* (L) and *Euschistus servus* (Say).

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SUMMARY

The scent gland of the rice stink bug *Oebalus pugnax* (F.) contains a complex mixture of chemicals which constitute the odoriferous substances ejected by this species. This mixture has two obvious liquid phases which separate after removal from the gland. The upper phase is a clear liquid which has been identified as primarily the hydrocarbon *n*-tridecane. The lower phase possesses the organoleptic characteristic of this species and contains *trans*-2-heptenal and at least six other components including an unsaturated dicarbonyl compound and at least one other monocarbonyl compound. Infrared data indicates that an alcohol and an acid are also present.

The mass spectral fragmentation patterns of the secretions from *Euschistus* and *Nezara* are very similar to that of *Oebalus*, indicating that the gross chemistry of their scent glands is similar. Vapor phase chromatographic anlyses of the secretions of the three genera indicate that the organoleptic differences found in the scent gland secretions of these pentatomids may be due to the ratios of different constituents.

RIASSUNTO

Sulla chimica del secreto odoroso di Pentatomidi.

La glandola dell'odore dell'Emittero *Oebalus pugnax* (F.) contiene una complessa miscela di sostanze odorose che costituiscono la secrezione espulsa da questa specie. Questa miscela ha due fasi liquide che si separano dopo che sono state tolte dalla glandola. La fase superiore è un liquido chiaro che è stato identificato soprattutto come idrocarburo *n*-tridecano. La fase inferiore possiede la caratteristica organolettica di questa specie e contiene *trans*-2-eptenale e almeno altri sei componenti compreso un componente dicarbossilico insaturo e almeno un altro componente monocarbossilico. Un esame a raggi infrarossi indica che sono presenti anche un alcool ed un acido.

Nel complesso gli spettri delle secrezioni di *Euschistus* e *Nezara* sono molto simili a quelli di *Oebalus* e ciò indica somiglianza di composizione chimica totale del secreto delle loro glandole odorifere. Analisi cromatografiche in fase di vapore delle secrezioni di tre generi indicano che differenze organolettiche trovate nelle secrezioni della glandola odorifera di questi Pentatomidi, possono essere dovute ai rapporti dei differenti costituenti.

CAVILL G. W. K., HINTERBERGER H. (*)

DOLICHODERINE ANT EXTRACTIVES

INTRODUCTION

Previous investigations of Dolichoderine ant extractives have resulted in the isolation of the following terpenoid compounds: iridomyrmecin (1, 2) (I), isoiridomyrmecin (2) (II), iridodial (2, 3) (III), methylheptenone (2, 3) (IV), and propyl isobutyl ketone (3) (V). Since then, the structure and reactions of the monoterpenes (I, II, and III), which are related to nepetalactone (4) (VI), have been considered in more detail (5, 6, 7, 8), and their stereochemistry elucidated (4, 7, 8, 9, 10). The unusual structural features of these compounds, together with their potential application as insecticides, have prompted synthesis (11, 12). Recently, the chemistry of these cyclopentanoid monoterpenes has been reviewed (9).

Although the *Dolichoderinae* have been displaced in many parts of the world, they remain a dominant sub-family in Australia. Current interest in insect secretions has prompted a survey of the extractives from these ant species, whence the isolation of the known terpenoid constituents (I-V), together with dolichodial [1] (VII) and 4-methylhexan-2-one (VIII), is now described from a wider range of *Dolichoderinae*.

EXTRACTIVES FROM *DOLICHODERUS* (*ACANTHOCLINEA*) *CLARKI* (WHEELER), *D.* (*ACANTHOCLINEA*) *DENTATA* (FOREL) AND *D.* (*DICERATOCLINEA*) *SCABRIDUS* (ROGER)

Dolichodial, a new cyclopentanoid monoterpene, is the major constituent, and methylhexanone, a minor constituent of *D.* (*Acanthoclinea*) *Clarki*, which has been collected in the Royal National Park, and the Kuringai Chase, near Sydney (13). The distillate from ants collected in the spring and summer months

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[1] The chemistry of dolichodial and related compounds has been presented in a separate communication.

contains dolichodial, together with a small amount of a second carbonyl compound. Ants collected in the winter, which give a much smaller yield of dolichodial, also contain an oxo-acid. These seasonal variations in the yield of dolichodial, and related compounds, from *D.* (*Achanthoclinea*) *Clarki*, are recorded in Table. I. Dolichodial has also been isolated from *D.* (*Acanthoclinea*) *dentata* (Forel), the ants being collected at Colo Vale, N. S. W., and Brindabella, A.C.T. (13).

TABLE 1.

Extractives of *Dolichoderus* (*Acanthoclinea*) *Clarki* (1)

Season	Quantity of ants (g)	Distillate	
		Yield (g)	Percentage of Body weight
Spring:			
September	282	1.05 (2)	
September	96	0.54	
October	376	0.85	
November	215	0.31	0.31
November	470	1.28	
November	210	0.87	
November	141	0.64	
Summer:			
December	431	0.67	
December	340	0.93	
January	468	0.92	
January	146	0.38	0.20
January	740	1.14	
February	173	0.25	
February	252	0.70	
Autumn:			
March	412	0.43	
April	124	0.10	
April	400	0.63	0.14
April	200	0.16	
April	400	0.81	
Winter:			
June	228	trace (3)	
August	910	1.04	0.11
August	670	0.92 (4)	

(1) Collected in the Royal National Park and the Kuringai Chase, near Sydney, from Winter, 1958, to Autumn, 1960.
(2) No acidic products were obtained from the crude extracts, collected during the months, September to April.
(3) Not examined for acidic constituents.
(4) Acidic products, 2,0 and 0,4 g, respectively, were also obtained.

Two varieties of *Dolichoderus* (*Diceratoclinea*) *scabridus* (Roger), of which the first has black, and the second, red legs, have been observed near Sydney. A colony of the « red-legged » variety, collected in the Bago Forest, N. S. W., gives methylheptenone and iridodial, but a second colony of this variety, from the Hawkesbury River, N.S.W., yields isoiridomyrmecin. A colony of the « black-legged » variety, collected in the Royal National Park, gives dolichodial. Such variations in the nature of the chemical extractives from different colonies of the same species of ant, do not appear to have been reported previously (13).

EXTRACTIVES FROM *IRIDOMYRMEX RUFONIGER* (LOWNE), *I. NITIDICEPS* (ANDRE), AND *I. MYRMECODIAE* (EMERY)

Iridomyrmex rufoniger is a common Australian ant. One colony, collected at Bankstown, N.S.W., gives methylheptenone and iridodial, whilst a second colony which was located near the first, yields dolichodial (13). The ants from each of these nests of *I. rufoniger* were collected on baits of raw meat, and it was noted that the baits laid in the vicinity of the nest entrance of a colony yielding methylheptenone and iridodial, were soon invaded by the larger meat ant, *I. detectus* (F. Sm.), which also contains methylheptenone and iridodial (2). Baits laid near the nest entrance of the colony yielding dolichodial were not invaded. It has been suggested previously that methylheptenone, from the meat ant, *I. detectus*, may be used in the laying of odour-trails (14). An additional example of the same variation in the extractives from *I. rufoniger*, has been noted for two colonies of these ants, at Cronulla, N.S.W., the nests being within fifty feet of each other.

Finally, *I. nitidiceps* (Andre), collected at Bago Forest, N.S.W., yields methylheptenone and iridodial, whilst *I. myrmecodiae* (Emery), collected at Honiara, British Solomon Islands, gives dolichodial. Table 2 summarises the sources of these structurally related terpenoid constituents (I-V, VII-VIII), which have now been reported from a wide range of the *Dolichoderinae*.

BIOGENESIS OF THE DOLICHODERINE ANT EXTRACTIVES

The pattern of structural relations which has resulted from these investigations of the Dolichoderine ant extractives, strongly supports the biogenetic path for the cyclopentanoid monoterpenes, originally proposed by Sir Robert Robinson (cf. 11). This path has been simulated in the recent synthesis of iridodial from L-(-)-citronellal (11).

Thus the biogenetic scheme presented for the Dolichoderine ant extractives is an extension of that proposed for the cyclopentanoid monoterpenes (11). Citral is suggested as the precursor for the ant extractives (cf. 15). Although not isolated from the *Dolichoderinae*, citral has been obtained from the mandibular glands of the « leaf-cutting » ant, *Atta sexdens* (16). Simple oxidation and

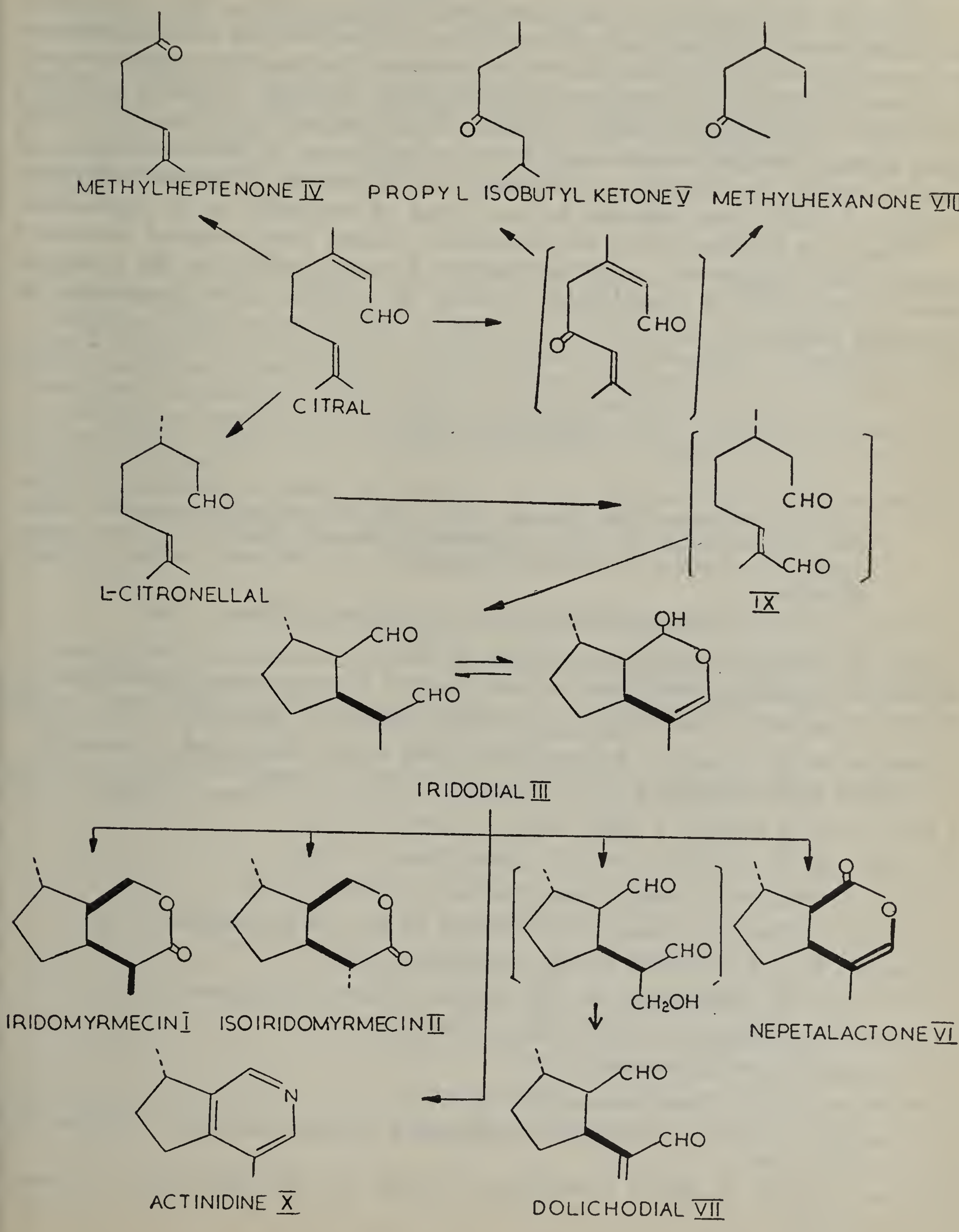
TABLE 2.

Dolichoderine ant extractives

Species	Compound	Reference
Iridomyrmex humilis	Iridomyrmecin ($C_{10}H_{16}O_2$)	1, 2
I. nitidus	Isoiridomyrmecin ($C_{10}H_{16}O_2$)	2, 7
I. detectus	Iridodial ($C_{10}H_{16}O_2$)	2, 8
I. conifer	and	2
I. nitidiceps	Methylheptenone ($C_8H_{14}O$)	13
	(a)	13
I. rufoniger	(b) Dolichodial ($C_{10}H_{14}O_2$)	13
I. myrmecodiae	Dolichodial	13
Tapinoma nigerrimum	Iridodial, Methylheptenone, Propyl isobutyl ketone ($C_8H_{16}O$)	3
Dolichoderus (Acanthoclinea) Clarki	Dolichodial, Methylhexanone ($C_7H_{14}O$)	13
D. (Acanthoclinea) dentata	Dolichodial	13
D. (Diceratoclinea) scabridus . . .	(a) Iridodial, Methylheptenone	13
	(b) Isoiridomyrmecin	13
	(c) Dolichodial	13

reduction processes, coupled with the reverse aldol reaction, would convert citral into the volatile ketones: methylheptenone, propyl isobutyl ketone, and methylhexanone (see Figure A). A stereospecific reduction of citral to L-citronellal, and then a terminal oxidation of the isopropylidene group in citronellal, would give 2,6-dimethyloct-2-en-1,8-dial (IX), which by the equivalent of the Michael condensation, would be converted into iridodial (11). Such terminal

FIGURE A


METHYLHEPTENONE IV
PROPYL ISOBUTYL KETONE V
METHYLHEXANONE VIII
CHO
CITRAL
CHO
CHO
L-CITRONELLAL
CHO
CHO
IX
CHO
CHO
OH
IRIDODIAL III
IRIDOMYRMECIN I
ISOIRIDOMYRMECIN II
CHO
CHO
CH₂OH
NEPETALACTONE VI
N
ACTINIDINE X
CHO
CHO
DOLICHODIAL VII

oxidations have been noted in the course of the metabolism of terpenoid compounds in animals (17).

Iridodial then occupies a key position in the biogenesis. The equivalent of a Cannizzaro reaction would convert it into iridomyrmecin and isoiridomyrmecin (cf. 8), and a β-hydroxylation of iridodial, followed by elimination of the elements of water, would give dolichodial. In addition, iridodial could be a precursor of the related plant products. Nepetalactone (4), from the catnip plant, *Nepeta cataria*, would result from an oxidation of the lactol form of iridodial, whilst the alkaloid, actinidine (18) (X), from *Actinidia polygama*, would be formed from iridodial by the action of ammonia, or its equivalent.

Whilst the Dolichoderine ant extractives present some unusual structural patterns, their biogenesis is explicable by the accepted path/s for the monoterpenes. It would be of considerable interest to test the above hypothesis in insect and plant.

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SUMMARY

Following upon previous studies in Italy and Australia, the isolation of the unusual terpenoid extractives: iridomyrmecin, isoiridomyrmecin (iridolactone), iridodial and dolichodial, together with the ketones: methylheptenone, methylhexanone and propyl isobutyl ketone, is now detailed from a wide range of Dolichoderine ants.

Variations are noted in the nature of the extractives from individual colonies of the same ant species. A biogenesis of the above cyclopentanoid monoterpenes, and of the related ketones, is discussed.

RIASSUNTO

Prodotti estrattivi da Formiche Dolicoderine.

Proseguendo studi precedenti condotti in Italia ed in Australia, l'isolamento di sostanze terpeniche insolite (iridomirmecina, isoiridomirmecina [iridolattone], iridodial e dolicodial) insieme a vari chetoni (metileptenone, metilesanone e propil-isobutil-chetone) è ora descritto dettagliatamente da un'ampia scala di formiche Dolicoderine.

Si notano variazioni nella natura dei prodotti estrattivi di singole colonie della stessa specie di formiche. Si discute la biogenesi dei monoterpeni ciclopentanoidi sopraddetti, e dei loro relativi chetoni.

BÜCHEL K. H., KORTE F. (*)

ZUR CHEMIE DER IRIDOLACTONE

Die argentinische Ameisenart *Iridomyrmex humilis* enthält bis zu einem Prozent des Körpergewichtes einen Kampfstoff, mit dem sie durch Auspritzen aus einer Hinterleibsdrüse andere Artgenossen abwehren und töten kann. Im Jahre 1948 gelang es M. Pavan, den Kampfstoff in kristalliner Form zu isolieren (1). Er nannte die Substanz nach der Ameisenart Iridomyrmecin. In weiteren Arbeiten konnte Pavan zeigen, dass dem Iridomyrmecin neben einer insektiziden Wirkung auch antibiotische Eigenschaften zukommen, so z.B. gegen *Typhus, Paratyphus, Cholera* und Tuberkelerregern (2). Fusco, Trave und Vercellone gelang um 1955 auf Grund von Abbaureaktionen die Strukturaufklärung des Iridomyrmecins (3). Ein wesentlicher Schritt war dabei der oxydative Abbau mit Kaliumpermanganat zu einer bekannten Nepetalsäure. Die Isolierung und Konstitutionsaufklärung vier verschiedener Nepetalsäuren, cis- und trans-Form sowie deren Epimere war kurz zuvor von McElvain und Eisenbraun durchgeführt worden. 1956 fanden Cavill und Lockley in der australischen Ameisenart Iridomyrmex nitidus ein weiteres Terpeno-Lacton, das sie durch Abbau zu einer Nepetalsäure vom Schmp. 81-82° als ein Epimeres des Iridomyrmecins erkannten (4).

Die epimeren Iridolactone lassen sich ineinander überführen. Durch Erhitezn einer Lösung von Iridomyrmecin in abs. $NaOCH_3$/Methanol erhält man zu 83 % Isoiridomyrmecin; Robinson und Mitarbeitern gelang die Überführung von Isoiridomyrmecin in Iridomyrmecin zu 37 % durch Erhitzen in Chinolin (5). Wir fanden, dass die gleiche Isomerisierung unter den Bedingungen der Gaschromatographie ebenfalls stattfindet (6). 1958 gelang Robinson und Mitarbeitern ausgehend von natürlichem d- und l-Citronellal die Darstellung von d- und l-Isoiridomyrmecin (7). Gleichzeitig wurden in unserem Bonner Laboratorium drei Totalsynthesen der Iridolactone entwickelt, die allgemeine Verfahren zur Synthese beliebig substituierter bicyclischer Lactone vom Typ der Iridomyrmecine darstellen (8).

Der durch Reformatzky-Reaktion von Methylcyclopentanon mit α-Brompropionsäure und anschliessender Wasserabspaltung erhaltene β, γ-ungesättigte

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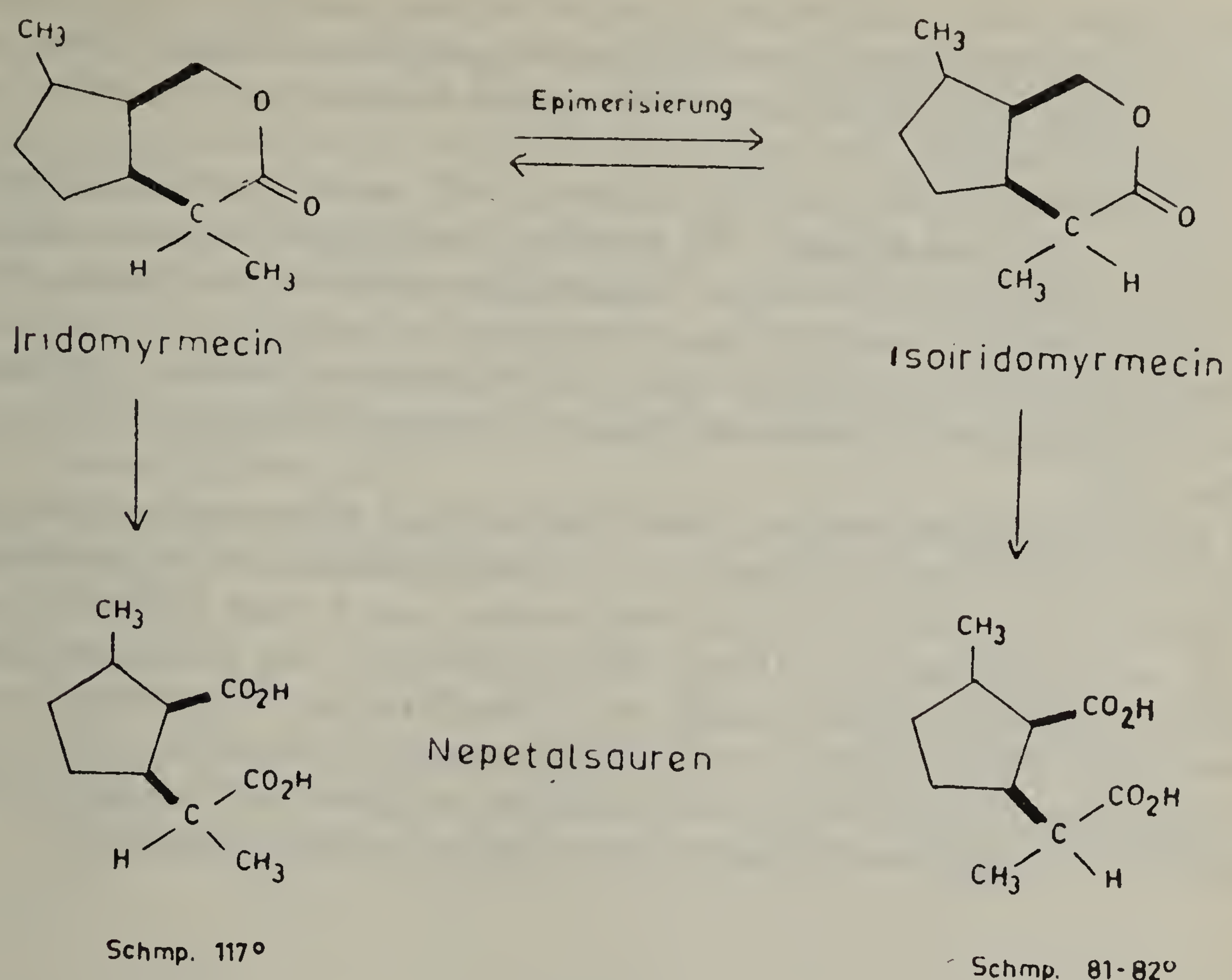
Epimerisierung
Iridomyrmecin
Isoiridomyrmecin
Nepetalsauren
Schmp. 117°
Schmp. 81-82°

Fig. 1

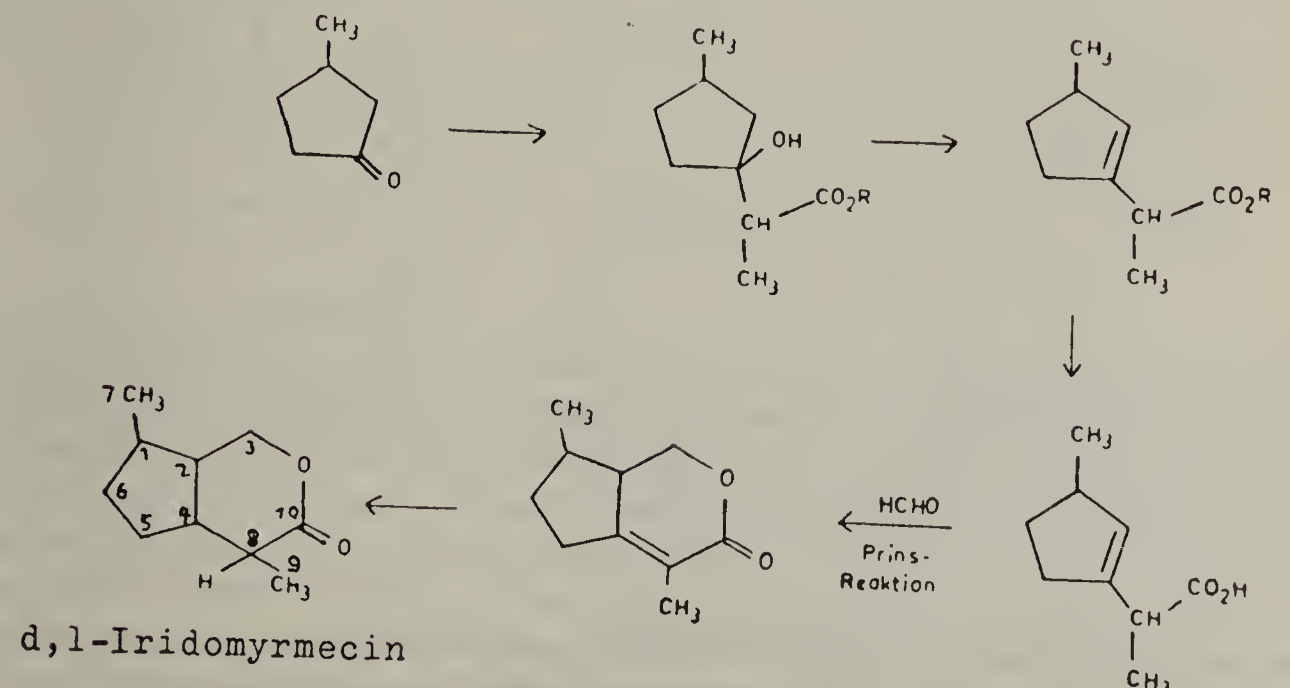
HCHO
Prins-
Reaktion
d,l-Iridomyrmecin

Fig. 2

Cyclopentenester wird nach Prins zu einem α, β-ungesätigtem bicyclischen Lacton umgesetzt. Dessen katalytische Hydrierung mit Raney-Nickel führt zu einem gesättigten Lacton, das mit dem von Pavan aus *Iridomyrmex humilis* isolierten Iridomyrmecin identisch ist.

Die Konfiguration der Methylgruppe an C_8 wird durch den letzten Reaktionsschritt, eine katalytische Hydrierung, festgelegt. Da eine katalytische Hydrierung erfahrungsgemäss eine einheitliche Cis-Addition darstellt, ist die Bildung eines sterisch einheitlichen Produktes verständlich. Das erhaltene d,l-Iridomyrmecin lässt sich zu 80 % in Methanol/$NaOCH_3$ epimerisieren, sodass mit Hilfe der Prins-Synthese grundsätzlich beide Epimere d,l-Iridolactone darstellbar sind.

Ein zweiter Syntheseweg zu den Iridolactonen geht aus von Methylcyclopentenaldehyd, der durch Oxydation von Methylcyclohexandiol gewonnen wird. Dabei bildet sich jedoch ein Isomerengemisch von 4- und 5-Methyl-cyclopentenaldehyd im Verhältnis 1:1. Durch Michaeladdition von Methylmalonester an den isomeren Aldehyd, Hydrierung der Aldeydgruppe, Verseifung und Decarboxylierung der Malonylgruppe und anschliessendem Ringschluss der gebildeten Hydroxysäure wurde ein isomeres Lactongemisch erhalten, das in der CH-Analyse und dem biologischen Verhalten dem Iridomyrmecin entspricht.

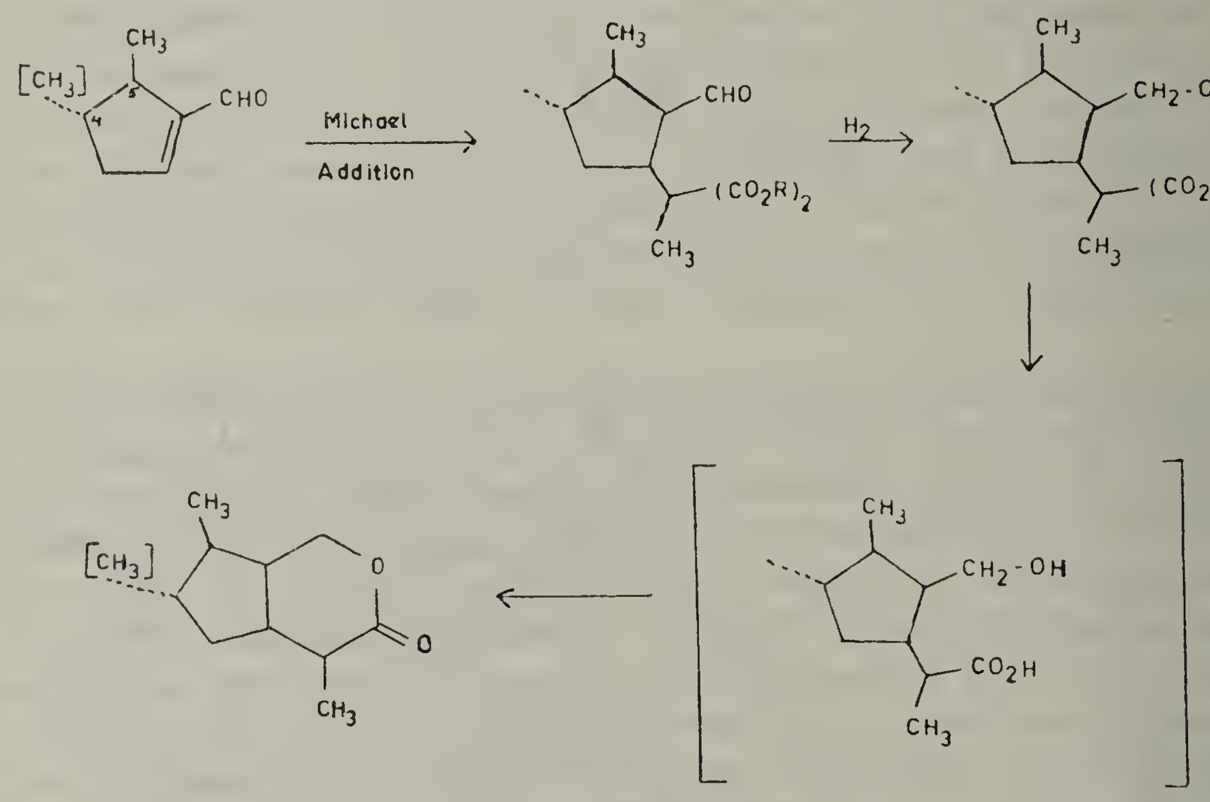

Fig. 3

Ein dritter Syntheseweg geht ebenfalls vom Methylcyclopentenaldehyd aus. Zunächst wurde wieder als Ausgangspunkt das Isomerengemisch des 5- und 4-Methylcyclopentenaldehyds gewählt. Durch Diels-Alder-Reaktion im Auto-

klaven bei 170° mit reinem cis-n-Propenyl-propyläther, der aus Propanol und Propionaldehyd leicht darstellbar ist, erhält man einen ungesättigten, cyclischen Acetal. Nach Hydrierung der Doppelbindung wird dieser mit verdünnter Schwefelsäure gespalten und mit Wasserstoffperoxyd in alkalischem Milieu zu einer Hydroxysäure oxydiert. Bei deren Destillation bildet sich sofort das bicyclische Lacton als ein farbloses, viskoses Öl. Das IR-Spektrum des Syntheseproduktes entspricht weitgehend dem des Isoiridomyrmecin, bis auf kleine Verschiebungen und eine zusätzliche Bande bei 1225 cm^{-1}. Iridomyrmecin entsteht nicht bei dieser Synthese, da es durch das Fehlen seiner charakteristischen IR-Bande bei 1280 und 1075 cm^{-1} — selbst im nicht fraktionierten Rohprodukt — sicher auszuschliessen ist.

Eine Auftrennung des Syntheseproduktes durch Destillation oder Gaschromatographie war nicht möglich.

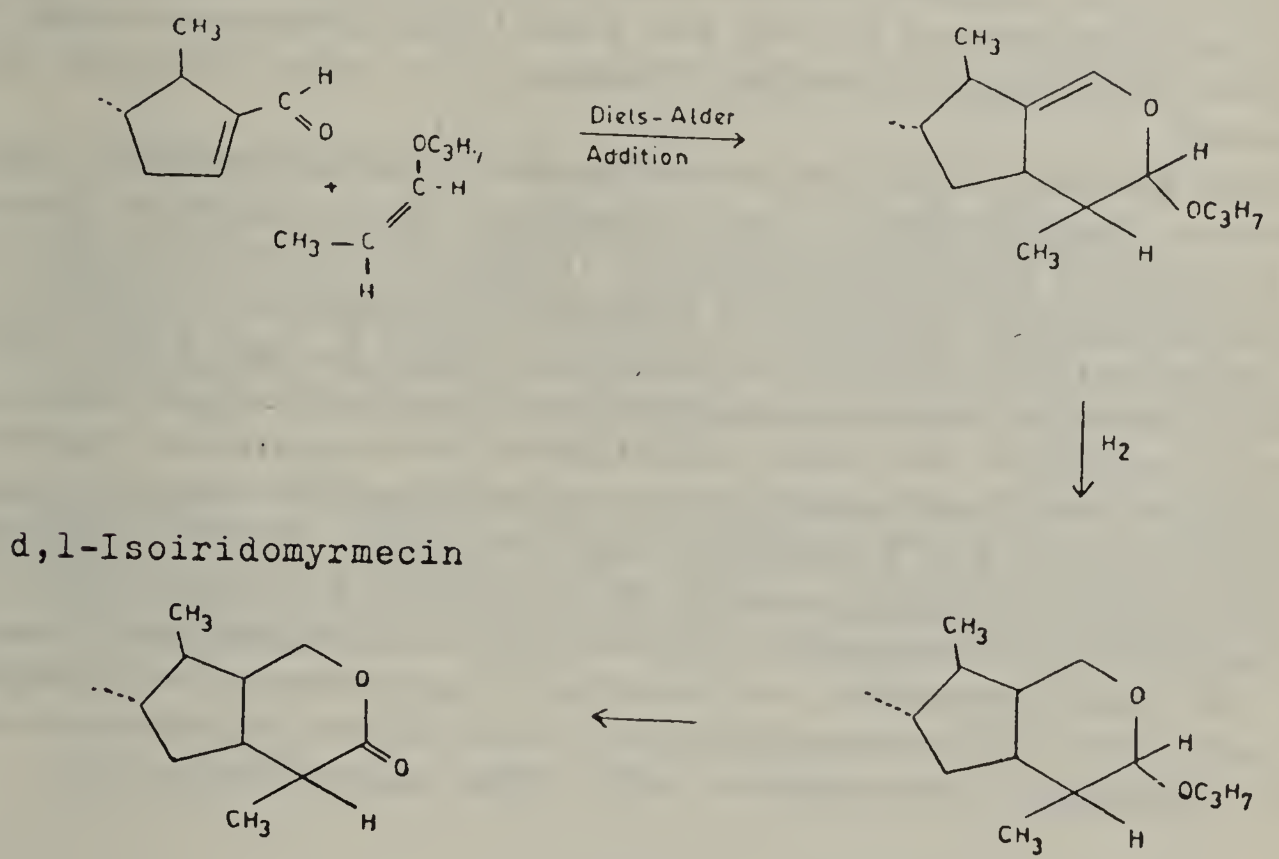

Fig. 4

Die Durchführung der Synthese mit reinem 5-Methylcyclopentenaldehyd sollte daher ein einheitliches Produkt liefern. Das Aldehydgemisch lässt sich jedoch durch Destillation nicht trennen, wie eine gaschromatographische Prüfung der Fraktionen ergab. Durch eine hintereinandergeschaltete präparative Gaschromatographie mit einem Beckmangerät GC 2, bei 130° Kolonne 3,4 m Silicon BRM, konnte jedoch eine Trennung erreicht werden. Der 4-Methylaldehyd zeigt im Gegensatz zu dem 5-Methylaldehyd im IR-Spektrum eine zusätzliche Bande bei 1225 cm^{-1}, die bei dem aus dem Aldehydgemisch erhaltenen Iridolacton schon als störend aufgefallen war.

Der reine 5-Methylaldehyd wurde nun nach der gleichen Synthese — wie vorher beschrieben — zum Iridolacton umgesetzt, das im IR-Spektrum mit natürlichem Isoiridomyrmecin (aus *Iridomyrmex nitidus* von Prof. Cavill isoliert) identisch war (9). Aus Petroläther konnte es in kristalliner Form vom Schmelzpunkt 32-33° gewonnen werden. Robinson gibt für das d, l-Isoiridomyrmecin den Schmelzpunk von 32-34° an. Die Konfiguration an C_8 wird bei der Diels-Alder-Addition des Cyclopentenaldehyds an den cis-Propenyl-propyläther festgelegt. Die Diels-Alder-Addition ist bekanntlich eine reine cis-Addition, wobei die Konfiguration des Dienophils erhalten bleibt (Alderregel). Die Bildung einer einheitlichen Konfiguration an C_8 ist daher verständlich.

Die gaschromatographische Prüfung des nach der Diels-Alder-Synthese dargestellten Lactons an verschiedenen Kolonnentypen und Füllungen (Silicon 550, Silicon BRM, Trikesylphosphat, Apiezon L) bei Temperaturen zwischen 160 und 180° C zeigt gleiche Retentionszeiten für das Syntheseprodukt und natürliches Isoiridomyrmecin, das uns von Prof. Cavill überlassen wurde. Unter den gleichen Bedingungen geprüftes Iridomyrmecin zeigt eine etwas kürzere Retentionszeit.

Bei dem Austesten der Bedingungen für die Geschromatographie wurde bei Temperaturen oberhalb 200° C eine Aufspaltung der registrierten Retentionsbanden beobachtet. Durch Erhöhung der Temperatur auf 240° konnte wieder eine einheitliche Bande, jedoch mit kürzerer Retentionszeit, erhalten werden. Durch eine präparative Gaschromatographie bei 240° konnte durch Isolierung und IR-Spektroskopie der chromatographierten Substanz nachgewiesen werden, dass sowohl natürliches wie auch synthetisches Isoiridomyrmecin unter diesen Bedingungen thermisch zu Iridomyrmecin epimerisiert. Die aufgefangenen Produkte kristallisierten aus Petroläther und entsprachen im IR-Spektrum und Schmelzpunkt dem d, l-Iridomyrmecin. Die thermische Epimerisierung bedeutet eine grundsätzliche Schwierigkeit für die gaschromatographische Charakterisierung von Isoiridomyrmecin, da auch bei Temperaturen von 160-180°, wo gerade noch eine Gaschromatographie der hochsiedenden Iridolactone möglich ist, eine geringfügige Isomerisierung nicht völlig auszuschliessen ist.

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ZUSAMMENFASSUNG

Lactone vom Typ des Iridomyrmecins erhält man auf folgenden 3 Wegen:

1) Cyclopenten-1-yl-propionsäuren-1 werden durch *Prins-Reaktion* und anschliessende Hydrierung in bicyclische δ-Lactone überführt. Auf diesem Wege wurde kristallines d,l-Iridomyrmecin synthetisiert.

2) An Cyclopentenaldehyde kann nach *Michael* Methylmalonester addiert werden. Nach Hydrierung der Aldehydgruppe erfolgt der Ringschluss zu den Iridolactonen über die Hydroxysäuren.

3) Durch *Diels-Alder-Reaktion* von cis-n-Propenyl-propyläther mit 5-Methyl-1-formyl-cyclopenten-1 (gereinigt durch präparative Gaschromatographie) erhält man einen ungesättigten, cyclischen Acetal, der-nach Hydrierung- mit H_2O_2 zum d,l,-Isoiridomyrmecin oxydiert wird.

Mit Hilfe der Gaschromatographie wurden die Syntheseprodukte auf Einheitlichkeit geprüft. Bei Temperaturen über 220° epimerisiert unter den Bedingungen der Gaschromatographie Isoiridomyrmecin in Iridomyrmecin.

RIASSUNTO

Contributo alla conoscenza della chimica degli iridolattoni.

Tre sono i modi per ottenere lattoni del tipo dell'iridomirmecina:

1) mediante una reazione di Prins e successiva idratazione si trasformano acidi ciclopenten-1-yl-propionici-1 in δ-lattoni; in tal modo fu sintetizzata d,l-iridomirmecina cristallina.

2) si può aggiungere all'aldeide ciclopentenica etere metil-malonico sec. Michael; dopo idratazione del gruppo aldeidico si ottiene la chiusura dell'anello degli iridolattoni attraverso gli ossiacidi.

3) mediante una reazione di Diels-Alder tra cis-n-propenil-propil-etere e 5-metil-1-formil-ciclopentene-1 (purificato mediante cromatografia gassosa preparativa) si ottiene un acetale insaturo ciclico che dopo idratazione viene ossidato a d,1,-isoiridomirmecina mediante H_2O_2.

Per mezzo della cromatografia gassosa si controllò l'identità dei prodotti sintetici. A temperatura superiore ai 220° nelle condizioni della cromatografia gassosa l'isoiridomirmecina epimerizza in iridomirmecina.

QUILICO A. (*), GRÜNANGER P. (*), PAVAN M. (**)

SUL COMPONENTE ODOROSO DEL VELENO DEL FORMICIDE *MYRMICARIA NATALENSIS* FRED.

I veleni dei Formicidi sono stati oggetto di numerose ricerche biologiche e chimiche. Proseguendo la serie di studi intrapresa da molti anni, in Congo abbiamo esaminato il comportamento in natura del Formicide *Myrmicaria natalensis* Fred. (subfam. *Myrmicinae*) riscontrando un attivo impiego del veleno nella lotta contro gli Insetti. Lo schiacciamento dell'operaia ha rivelato la presenza nel corpo di un prodotto avente caratteristico odore terpenico; la dissezione del corpo ha permesso di mettere in evidenza che l'odore proveniva dal serbatoio contenente il veleno impiegato dall'operaia per offesa e difesa; saggi preliminari hanno permesso di conoscere la solubilità della sostanza nei vari solventi. Su queste tracce iniziali abbiamo sviluppato la successiva ricerca chimica del costituente odoroso del veleno, che è stata condotta su materiali raccolti direttamente da uno di noi e preparati nei solventi adatti (ad es. etere etilico) e su materiali fatti raccogliere e conservare con semplice aggiunta di NaCl in polvere.

Dal frazionamento dell'estratto etereo e dall'estrazione dei corpi conservati con NaCl, sono stati ottenuti prodotti identici, rispondenti alla sostanza odorosa riscontrata nel vivo: essi sono l-limonene (20 %), d-limonene (80 %). La determinazione chimica è stata ottenuta attraverso la formazione di vari derivati, con gli spettri I. R. ed U. V. e con l'analisi cromatografica in fase di vapore, operando parallelamente su campioni autentici di d-limonene e l-limonene. Il ritrovamento di questi due limoneni costituisce il primo caso della presenza di questi idrocarburi terpenici in organismi animali; esso viene ad allargare la conoscenza delle sostanze di natura terpenica componenti delle secrezioni dei *Formicidae* di cui riportiamo l'elenco nella Tabella 1.

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Tabella 1

Sostanze di natura terpenica nei Formicidae	ORIGINE g.v. = glandole velenifere g.a. = « glandole anali » g.m. = glandole mandibolari		Letteratura
iridomirmecina	*Iridomyrmex humilis* Mayr	g.a.	3, 5, 6, 7
isoiridomirmecina	*Iridomyrmex nitidus* Mayr	g.a.	2, 5, 6, 7
iridodial (1)	*Iridomyrmex detectus* Sm.	g.a.	2, 5, 6, 7
	I. conifer For.	g.a.	2, 5, 6, 7
	I. nitidiceps	g.a.	2
	Tapinoma nigerrimum Nyl.	g.a.	8, 12
dolicodial	*Dolichoderus, Iridomyrmex*	g.a.	2
dendrolasina	*Lasius (Dendrolasius) fuliginosus* Latr.	g.m.	9, 10
citrale	*Atta sexdens rubropilosa* For.	g.m.	1
citronellal	*Acanthomyops* sp.	g.m.	11
citronellolo	» »	g.m.	11
d-limonene	*Myrmicaria natalensis* Fred.	g.v.	4
l-limonene	» » »	g.v.	4

Con l'individuazione dei due limoneni quali costituenti del secreto delle glandole velenifere non si esaurisce lo studio del veleno.

Nell'esame dei prodotti ricavati dall'estratto etereo totale e dalla distillazione dei corpi conservati con NaCl, sono stati riconosciuti presenti acido acetico, ac. propionico, ac. isobutirrico, ac. isovalerianico, ma non è possibile per ora precisare quale origine nel corpo dell'animale possano avere queste sostanze. Le ricerche continueranno per l'ulteriore studio chimico anche di altri prodotti messi in evidenza negli estratti ma non ancora chimicamente definiti.

(1) Presente anche in una specie del gen. *Dolichoderus* dell'Australia. Un'altra dialdeide è la nuova sostanza $C_{10}H_{16}O_2$ denominata dolicodial (in *Dolichoderus, Iridomyrmex*, dell'Australia) segnalata da Cavill (2).

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RIASSUNTO

Il veleno della Formica *Myrmicaria natalensis* Fred. del Congo contiene sostanze odorose che sono state identificate in *l*-limonene e *d*-limonene, con forte prevalenza (80 %) della forma *d*. Dall'estratto etereo totale dei corpi di *M. natalensis* sono stati inoltre isolati nelle rispettive proporzioni: acido acetico (35 %); acido propionico (22 %); acido isobutirrico (tracce); acido isovalerianico (31 %).

SUMMARY

On the odorous component of the poison of Myrmicaria natalensis *Fred.* (Formicidae).

Myrmicaria natalensis Fred. (Subfam. *Myrmicinae*) from the Congo, produces an smelly poison. From its poison *l*, *d*-limonene have been isolated, with a great prevalence (80 %) of *d*-form.

From the whole ether-extract have been isolated too: acetic acid (35 %); propionic acid (22 %); isobutirric acid (some traces); isovalerianic acid (31 %).

HECKER E. (*)

CHEMIE UND BIOCHEMIE DES SEXUALLOCKSTOFFES DES SEIDENSPINNERS (*BOMBYX MORI* L.)

Auf dem X. Internationalen Entomologenkongress in Montreal konnten wir über den Test und die Isolierung von wenigen Milligrammen des Sexuallockstoffes des Seidenspinners (*Bombyx mori* L.) aus 300.000 Duftdrüsen berichten (1). Die Substanz wurde als 4'-Nitro-azobenzol-carbonsäure-(4)-ester isoliert, war jedoch noch nicht vollkommen rein und zeigte als auffallendes Charakteristikum eine intensive UV-Absorption bei 231 mμ. Der Aufarbeitungsgang ist inzwischen wiederholt und in vielen Teilen verbessert worden.

Als Ausgangsmaterial dienten die Duftdrüsen von 500.000 weiblichen Seidenspinnern. Nach Extraktion des zerkleinerten Drüsenmaterials mit einem Alkohol-Äther-Gemisch und Verdampfen des Lösungsmittels verblieben 280 g des schwach gelb gefärbten Rohextraktes, der das wirksame Prinzip enthält. In mehreren Reinigungsschritten konnten daraus 3,4 g einer wachsartigen Fraktion gewonnen werden, die nur noch aus den Alkoholen des Drüsenmaterials besteht und die die Lockstoff-Einheit (L. E.) (2) in 10^{-4} γ/ml enthält. Dies entspricht der anfänglich vorhandenen Wirkstoffmenge. Die im Trennungsgang entfernten Begleitsubstanzen sind im Test praktisch nicht wirksam.

Die von Sterinen weitgehen befreite Alkoholfraktion wurde mit 4'-Nitro-azobenzol-carbonsäure-(4)-chlorid verestert. Das erhaltene Gemisch von 5,6 g der 4'-Nitro-azobenzol-carbonsäure-(4)-ester wurde durch fraktionierte Fällung aus wässrigem Aceton in drei Fraktionen A, B und C aufgeteilt, die nach Verseifung im Test ähnliche biologische Wirksamkeit zeigten. Aus diesen Fraktionen konnten nach Verteilungschromatographie auf hydrophobierter Kieselgur und Umkristallisieren 12 mg des reinen 4'-Nitro-azobenzol-carbonsäure-(4)-esters des Sexuallockstoffes mit einem Schmelzpunkt von 95-96° isoliert werden (3). Der Ester lässt sich zum Lockstoffalkohol verseifen, der die Lockstoffeinheit in 10^{-10} γ/ml enthält. Die Analyse des Esters ebenso wie das aus seiner UV-Absorption bei 331 mμ errechnete Molekulargewicht von 475 ± 15 stimmen

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gut auf einen zweifach ungesättigten Alkohol $C_{16}H_{30}O$ für den Sexuallockstoff. Das Infrarotspektrum weist darauf hin, dass die beiden Doppelbindungen in cis-trans-Konfinguration vorliegen.

Zur Konstitutionsaufklärung wurden 2 mg des durch Verseifung erhältlichen Lockstoffalkohols der katalytischen Hydrierung unterworfen. Sie lieferte Cetylalkohol, womit bewiesen ist, dass der Sexuallockstoff ein geradkettiges Hexadeka-dienol sein muss. Die Lage der Doppelbindungen wurde durch eine Methode ermittelt, die darauf beruht, dass sich die 4'-Nitro-azobenzol-carbonsäure-(4)-ester ungesättigter Alkohole auch in sehr kleinen Mengen mit Kaliumpermanganat in Aceton zu definierten Bruchstücken spalten lassen (4). Das speziell zu diesem Zweck entwickelte Mikroverfahren liefert mit 1 mg des Lockstoffderivates Buttersäure, Oxalsäure und den 4'-Nitro-azobenzol-carbonsäure-(4)-ester der ω-Hydroxy-caprinsäure. Damit sind sämtliche 16 Kohlenstoffatome des Lockstoffalkohols erfasst und die Doppelbindungen müssen, dem Ergebnis der Spaltung entsprechend, zwischen C_{10} und C_{11} sowie C_{12} und C_{13} liegen.

Der erste geschlechts- und artspezifische Sexuallockstoff, der in reinem Zustand isoliert und dessen Struktur aufgeklärt werden konnte, ist damit als Hexadeka-dien-10,12-ol-(1) charakterisiert (3).

Wegen der im Molekül enthaltenen 2 Doppelbindungen sind theoretisch insgesamt 4 geometrisch isomere Hexadeka-dien-10,12-ole-(1) möglich, die sich durch die Anordnung der Wasserstoffatome an den Doppelbindungen unterscheiden. Welches der 4 Isomeren der natürliche Lockstoff ist, kann mit physikalischen und chemischen Methoden allein nicht sicher entschieden werden. Das Infrarot-Spektrum weist darauf hin, dass der Naturstoff mit einem der beiden möglichen cis, trans-Isomeren identisch ist. Eine eindeutige Entscheidung dieser Frage kann nur durch Synthese der Isomeren und Austestung ihrer biologischen Wirksamkeit herbeigeführt werden. Wir haben daher alle vier geometrischen Isomeren Hexadeka-dien-10,12-ole-(1) auf verschiedenen Wegen dargestellt, auf die hier im einzelnen nicht eingegangen werden kann.

Tab. 1

Die biologische Wirksamkeit der synthetisch dargestellten, geometrisch isomeren Hexadeka-dien-10,12-ole-(1).

Verbindung	Schmp.	(**) λ max	(**) max	Biol. Wirks. L.E. γ/ml
trans, trans-	36 - 37°	230,5	30600	1
10-trans, 12-cis-	flüssig (*)	231	21900	10^{-13}
10-cis, 12-trans-	flüssig (*)	232,5	25850	10^{-3}
cis, cis-	25,5 - 26,5	235	27500	10

(*) bei 20° C.
(**) in Äthanol.

Die biologische Wirksamkeit der synthetischen Produkte ist in Tab. 1 wiedergegeben. Das 10-trans-12-cis-Isomere vermag im Test die typische Verhaltensreaktion noch in einer Verdünnung von 10^{-13} γ/ml auszulösen. Demgegenüber ist das 10-cis, 12-trans-Isomere mit einer Lockstoffeinheit von 10^{-3} γ/ml 10^{10} mal weniger wirksam, während das cis-cis- und das trans-trans-Isomere noch unwirksamer sind. Die hohe biologische Wirksamkeit des 10-trans, 12-cis-Isomeren ist im Rahmen der Genauigkeit des Verhaltenstestes mit der Wirksamkeit des Naturstoffes vergleichbar und seine chemischen und physikalischen Daten zeigen, das die Verbindung mit dem natürlichen Sexuallockstoff vollkommen identisch ist. Der Sexuallockstoff des Seidenspinners ist danach das 10-trans-12-cis-Hexadekadienol-(1), der erste Sexuallockstoff, dessen Chemie vollständig bekannt ist und der synthetisch hergestellt werden kann.

Da der Sexuallockstoff des Seidenspinners in beliebigen Mengen zugänglich ist, ergeben sich interessante biologische Aspekte, von denen nur einer gestreift werden soll. Die hohe Artspezifität der Sexuallockstoffe ist schon seit langem bekannt. Wie aus Tab. 1 hervorgeht, sind selbst die dem natürlichen Sexuallockstoff am nächsten verwandten Isomeren vergleichsweise unwirksam. Man wird daher nicht fehlgehen mit der Annahme, dass die hohe Artspezifität der Sexuallockstoffe auf einer hohen Strukturspezifität der Rezeptoren auf der Schmetterlingsantenne beruht. Die Lösung des chemischen Problems führt damit unmittelbar hin zu aktuellen biologischen Fragen, von deren Bearbeitung wir weitere interessante Ergebnisse erhoffen.

Ausser dem Vortragenden waren an den beschriebenen Experimenten beteiligt: Die Herren Dr. Beckmann und Dr. Stamm, Max-Planck-Institut für Biochemie, an der Strukturaufklärung und Isolierung, die Herren Dr. Eiter und Dr. Truscheit, Wissenschaftliches Hauptlaboratorium der Farbenfabriken Bayer, Leverkusen, sowie Herr W. Koch und Herr M. Hopp Max-Planck-Institut für Biochemie, an der Synthese der 4 geometrischen Isomeren.

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ZUSAMMENFASSUNG

Aus den Lipoidanteilen von 500.000 Duftdrüsen weiblicher Seidenspinner liessen sich 12 mg des reinen 4'-Nitroazobenzol-carbonsäure-(4)-esters des Sexuallockstoffs (F. 94-96°) isolieren. Der Ester kann zum Lockstoffalkohol verseift werden, der die Lockstoffeinheit in 10^{-10} γ/ml enthält. Analyse und Molekulargewichtsbestimmung des Esters stimmen auf einen

zweifach ungesättigten Alkohol $C_{16}H_{30}O$ und das UV-Spektrum beweist das Vorliegen konjugierter Doppelbindungen. Katalytische Hydrierung des freien Lockstoffalkohols und oxydative Spaltung des 4'-Nitro-azobenzol-carbonsäure-(4)-esters ergaben, daβ ein Hexa-deka-dien-10,12-ol-(1) vorliegt. Das IR-Spektrum weist auf cis, trans-Konfiguration der konjugierten Doppelbindungen hin. Es wird durch Synthese und biologischen Test gezeigt, daβ der Sexuallockstoff ein 10-trans-12-cis-Hexadekadienol-(1) ist.

RIASSUNTO

Chimica e biochimica della sostanza attrattiva sessuale del Baco da seta (Bombyx mori *L.*)

Dalle frazioni lipoidiche di 500.000 glandole odorifere di femmine di Baco da seta si poterono isolare allo stato puro 12 mg di estere dell'acido 4'-nitroazobenzol-carbossilico-(4) della sostanza attrattiva sessuale (F. 94-96°). L'estere può venir saponificato ad alcool, che contiene l'unità attrattiva in dose di 10^{-10} gamma/ml. Analisi e determinazione del P.M. dell'estere indicano un alcool due volte insaturo $C_{16}H_{30}O$ e lo spettro ultravioletto indica la presenza di doppi legami coniugati. L'ossidazione catalitica dell'alcool libero e la demolizione ossidativa dell'estere dell'acido 4'-nitroazobenzol-carbossilico-(4) dimostrarono trattarsi di un esa-deca-dien-10,12-olo-(1). Lo spettro infrarosso indica una configurazione cis, trans dei doppi legami coniugati. Si dimostra mediante sintesi e test biologici che la sostanza attrattiva è un 10-trans-12-cis-esadeca-dien-olo-(1).

TRAVE R. (*), GARANTI L. (*), PAVAN M. (**)

SUL SECRETO DELLE GLANDOLE MANDIBOLARI DELLA LARVA DI *COSSUS COSSUS* L. (*C. LIGNIPERDA* FABR.) (*LEPIDOPTERA*)

INTRODUZIONE

E' noto che in numerose specie di Lepidotteri le larve producono secrezioni particolari alcune delle quali hanno formato oggetto di studi chimici. Citiamo ad esempio le ricerche di Poulton 1886, 1887 su *Cerura vinula* L. (*Dicranura vinula*) e di Denham 1888 sulla larva di *Notodonta concinna*.

Uno dei casi più noti è quello di *Cossus cossus* L. la cui larva vivente nel tronco di varie essenze (pioppo, salice, ec.) produce una sostanza intensamente odorosa che viene emessa attraverso gli orifizi di sbocco del dotto escretore delle glandole mandibolari, il cui secreto è contenuto in due grandi serbatoi cilindrici decorrenti parallelamente alla porzione anteriore del tubo digerente.

La nostra attenzione su tale secrezione è stata richiamata anche dal fatto che secondo Henseval (1897) la sostanza contiene il 10 % di zolfo; tale Autore afferma inoltre che non è saponificabile, ha carattere insaturo probabilmente aromatico e un tenore di C = 76,6 % e di H = 11,01 %.

Nella letteratura si registra inoltre che il secreto ha una certa tossicità per la mosca; è attivo su una specie di *Oospora* parassita di insetti; non ha alcuna influenza sul legno per cui sarebbe da escludersi un suo impiego quale ausiliare nell'attacco delle fibre legnose durante lo scavo della galleria.

La nostra ricerca è consistita nella verifica ed estensione dei dati chimici e biologici noti. In questa nota si riportano riassuntivamente alcuni risultati delle nostre ricerche che sono ancora in corso (1).

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(1) Si ringraziano i colleghi Dott. A. Baggini, M. Miradoli-Zatti, G. Ronchetti, M. L. Valcurone per l'aiuto prestatoci nel corso di queste ricerche.

MATERIALE

Il secreto puro è stato ottenuto dagli animali dissezionati in narcosi, prelevando i serbatoi, asciugandoli esternamente dall'emolinfa su carta bibula e raccogliendo il liquido interno o con aspirazione in siringa o facendolo sgocciolare in fiala.

PRIMI RISULTATI DELLA RICERCA CHIMICA

Contrariamente alle affermazioni di Henseval le analisi hanno messo in evidenza che nel secreto non si trova zolfo. Il secreto è risultato composto da una miscela di 3 componenti molto difficilmente separabili per distillazione frazionata. Con l'analisi cromatografica in fase di vapore i tre componenti risultarono nella proporzione di 50 - 40 - 10 %.

Il secreto ha una formula bruta $C_{16}H_{28}O_2$, corrispondente ad un alcool doppiamente insaturo. L'insieme delle ricerche finora compiute permette di ritenere che deve considerarsi costituito per la maggior percentuale di una miscela di acetati di alcooli C_{14} primari, a catena lineare, insaturi; il componente o i componenti più abbondanti posseggono due doppi legami separati da 8 atomi di carbonio. Le ricerche continuano per stabilire la posizione dell'aggruppamento $-CH=CH(CH_2)_6CH=CH-$ e chiarire completamente la struttura dei vari componenti. Poichè non risultavano descritti nella letteratura chimica nè alcooli C_{14}, doppiamente insaturi con la medesima posizione dell'insaturazione, nè i corrispondenti acetati, abbiamo denominato *cossina A*, *cossina B*, *cossina C* i tre componenti principali: col solo nome *cossina* intendiamo indicare la miscela naturale dei tre componenti.

PRIMI RISULTATI DELLA RICERCA BIOLOGICA

Abbiamo approfondito per ora soprattutto la ricerca dell'attività insetticida della cossina per contatto e per respirazione. Le prove per contatto sono state condotte in scatole Petri smerigliate, sperimentando in comparazione con uguali dosi di DDT-pp'. Queste prove a 100 gamma/cm^2 hanno rivelato che sulle 13 specie di Blattoidei, Isotteri, Ortotteri, Emitteri, Lepidotteri e Coleotteri (1) da noi sperimentate, la cossina in genere è priva di attività o in qualche caso ha attività ridottissima rispetto al DDT-pp', mentre su 8 specie di Imenotteri Formicidi (2) esercita un'attività notevole, generalmente superiore a quella del DDT-pp', salvo su *Formica lugubris* e su *Lasius* (*Dendrolasius*) *fuliginosus* sulle quali ha una tossicità molto bassa.

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(1) *Pycnoscelus surinamensis* L., *Blatta orientalis* L., *Blattella germanica* L., *Reticulitermes lucifugus* Rossi, *Kalotermes flavicollis* F., *Acheta domestica* L., *Locusta migratoria migratorioides* R. e F., *Oncopeltus fasciatus* Dallas, *Ephestia kuhniella* Zell., *Tenebrioides mauritanicus* L., *Calandra oryzae* L., *Tenebrio molitor* L., *Tribolium confusum* Duv.

(2) *Tetramorium caespitum* L., *Leptothorax acervorum* L., *Crematogaster scutellaris* Oliv., *Messor structor* Latr., *Iridomyrmex humilis* Mayr, *Tapinoma nigerrimum* Nyl., *Formica lugubris* Zett., *Lasius (Dendrolasius) fuliginosus* Latr., *Camponotus aethiops* Latr.

Per ora non ci è possibile indicare a quale dei tre componenti sia dovuta l'attività biologica .

Tutte le prove dell'attività tossica dei vapori di cossina su 10 specie di Insetti, comprese 5 specie di Formicidi, hanno dimostrato attività nulla o insignificante in confronto a pari dosi (cc 100/m^3) di solfuro di carbonio.

CONCLUSIONI

In questa ricerca tuttora in corso abbiamo dunque ottenuto una rettifica dei dati di Henseval 1897 che attribuiva alla molecola di cossina la presenza di zolfo (mentre manca in tutti i campioni da noi esaminati) ed una struttura ciclica: risulta invece che i tre componenti cossina A, cossina B, cossina C sono acetati di alcooli primari, insaturi, lineari, rispondenti alla formula bruta $C_{16}H_{28}O_2$.

Abbiamo anche trovato un'attività tossica della cossina su varie specie di Formicidi mentre a pari dosi varie specie di Insetti di altri ordini non manifestano reazioni di tossicità. Benchè la maggiore attività tossica si verifichi verso quegli Insetti che sono i più probabili frequentatori delle gallerie scavate nei tronchi dalle larve di *Cossus cossus*, allo stato attuale delle nostre conoscenze non si può dire se il secreto possa essere interpretato come un veleno selettivo verso gli avversari più probabili.

Le ricerche proseguono sia per ottenere la completa conoscenza chimica, sia per definire le proprietà biologiche dei vari componenti la cossina e il suo significato in natura.

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RIASSUNTO

Viene studiata la natura chimica del secreto delle glandole mandibolari di *Cossus cossus* L. (*ligniperda* F.) costituito da un olio incolore, di odore caratteristico, che si comporta alla distillazione a pressione ridotta come un composto unitario, ma che alla cromatografia in fase di vapore si rivela invece consistere di tre prodotti diversi presenti nelle proporzioni di circa 50-40-10 % rispettivamente e con ogni probabilità isomeri tra loro. Indichiamo i tre prodotti coi nomi cossina A, cossina B, cossina C. Col nome cossina indichiamo il secreto grezzo.

La miscela all'analisi e alla determinazione del peso molecolare risponde alla formula bruta $C_{16}H_{28}O_2$.

Dall'insieme dei risultati finora ottenuti risulta che la cossina è una miscela di acetati di alcooli a 14 atomi di carbonio, lineari, primari, doppiamente insaturi: essi si differenziano per la posizione dei doppi legami. La parte di struttura finora conosciuta comprende il gruppo $—CH{=}CH(CH_2)_6CH{=}CH—$.

La cossina può forse essere interpretata come sostanza di difesa contro le Formiche poichè su queste ha azione tossica per contatto più forte che sulle altre specie di Insetti saggiati. Fra gli Insetti dell'*habitat* delle larve di *Cossus cossus* (tronchi di piante di varie specie) le Formiche sono quelle che hanno maggiori probabilità di penetrare nei tronchi e causare disturbo alle larve.

SUMMARY

On the secretion from the mandibular glands of Cossus cossus *L. larva* (Lepidoptera).

The chemical nature of the secretion of the mandibular glands of *Cossus cossus* L. (*ligniperda* F.) is studied: it consists of a colourless oil that has a characteristic smell and that acts at reduced pressure distillation as a single compound, but that at the cromatography in vapour phase, shows itself to consist of three different compounds, present respectively in the proportion of about 50, 40, 10 %, and which are probably isomeric among themselves. We give the three compounds the names: *cossina* A, *cossina* B, *cossina* C. We use the name *cossina* by itself to imply the crude secretion. On the analysis and determination of it's molecular weight the mixture has the gross formula $C_{16}H_{28}O_2$.

From the collective results one can conclude that the cossina is a mixture of linear, primary, doubly insaturate alcohol acetates, having 14 atoms of carbon, which differ in the position of their double links. The structural part which is at the moment known comprises the group $—CH{=}CH(CH_2)_6CH{=}CH—$.

The cossina can perhaps be interpreted as a defense substance against Ants since on these it has a toxic action stronger than on other insects experimented on. Among the insects from the *habitat* of the *Cossus cossus* larvae (stems of plants of various species) Ants are those which have the greatest probability of penetrating the stems and disturbing the larvae.

REMBOLD H. (*)

ÜBER DEN WEISELZELLENFUTTERSAFT DER HONIGBIENE

Die Determinierung der aus einem befruchteten Ei stammenden Bienenlarve zur Königin erfolgt schon bei den 1-2 Tage alten Tieren durch den sogenannten Weiselzellenfuttersaft (Gelée royale, Royal Jelly). Er ist ein Kopfdrüsensekret junger Arbeitsbienen, mit dem die Königinnenlarven in überreichlichem Masse gefüttert werden und das man deshalb aus den Königinnenzellen in Mengen von bis zu 300 mg gewinnen kann. Die Zellen der Arbeiterinnenbrut enthalten demgegenüber einen nur sehr geringen Überschuss von etwa 5 mg an Arbeiterinnenfuttersaft.

Es ist bis jetzt noch nicht gelungen, eintägige Bienenlarven mit einem synthetischen Futter aufzuziehen, um dann durch Zusatz von Weiselfutterfraktionen einen biologischen Test zum Nachweis des determinierenden Prinzips durchzuführen. Wir beschäftigen uns deshalb parallel zu solchen Versuchen mit der chemischen Charakterisierung des Weiselzellenfutters, um Aufschluss über seine Zusammensetzung und, durch Vergleich mit Arbeiterinnenfuttersaft, über seine charakteristischen Bestandteile zu erhalten.

Der Weiselzellenfuttersaft ist eine gelbliche, milchig trübe Masse mit einem Wassergehalt von durchschnittlich 60 %. Eine grobe Auftrennung der Trockensubstanz (Tab. 1) ergibt mit 10 % einen relativ hohen Gehalt an ätherlöslichem

TAB. 1.
Zusammensetzung des Weiselzellenfuttersaftes

A. WASSER	60 %		
B. TROCKENSUBSTANZ	40 %		
1. *Lipide*		10 %	
a.) stark saurer Anteil			90 %
b.) schwach saurer Anteil			2 %
c.) Neutralanteil			8 %
2. *Dialysierbares Material*		52 %	
Zucker, Aminosäuren, Vitamine etc.			
3. *Nicht dialysierbares Material*		38 %	
a.) in Wasser löslich			55 %
b.) in Wasser unlöslich			45 %

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Material, das sich fast vollständig in Natronlauge löst, also aus freien Fettsäuren besteht. Den Hauptanteil der Trockensubstanz bilden mit etwa 52 % niedermolekulare, dialysierbare Stoffe, vor allem Zucker, Aminosäuren und Vitamine. Die nicht dialysablen Bestandteile sind zur Hauptsache in Wasser lösliche bzw. unlösliche Proteine.

Aus der Lipoidfraktion haben wir eine Fettsäure isoliert und in ihrer Struktur geklärt (1):

$$HO\,H_2C—CH_2—(CH_2)_5—\overset{H}{C}=\overset{H}{C}—COOH$$

$$H_3C—CO—(CH_2)_5—\overset{H}{C}=\overset{H}{C}—COOH$$

Diese 10-Hydroxy-decen-2-säure ist der Hauptbestandteil der freien Fettsäuren im Weiselzellenfuttersaft; eine vergleichende Untersuchung von Arbeiterinnenfuttersaft ergab, dass sie auch hier in der gleichen Konzentration enthalten ist. Über die physiologische Bedeutung dieser Fettsäure, die bis jetzt nur bei der Honigbiene nachgewiesen werden konnte, wissen wir noch nichts; sie dürfte wegen ihrer bakteriostatischen und bakteriziden Eigenschaften vor allem für die Haltbarkeit der Futtersäfte verantwortlich sein. Auf ihre Ähnlichkeit mit der « Queen substance », einer 9-Oxo-decen-2-säure, deren Struktur kürzlich von Callow und Johnston (2) sowie von Barbier und Lederer (3) geklärt wurde, sei in diesem Zusammenhang hingewiesen: beide Fettsäuren kommen in der Mandibulardrüse der Königin, die Decenolsäure in der Mandibulardrüse und gleichzeitig in geringer Konzentration auch in der Pharyngealdrüse (4) der Arbeiterin vor.

Einen Vergleich des Vitamingehalts von Weisel- und Arbeiterinnenfuttersaft zeigt Tab. 2. Beide Futtersäfte sind in ihrem Vitamingehalt sehr ähnlich. Eine Ausnahme bilden die Pantothensäure, die im Weiselfutter in etwa zehnfacher, und die Folsäure, die in etwa doppelter Konzentration im Vergleich zum Arbeiterinnenfutter enthalten sind (5). Diese beiden Vitamine dürften vor

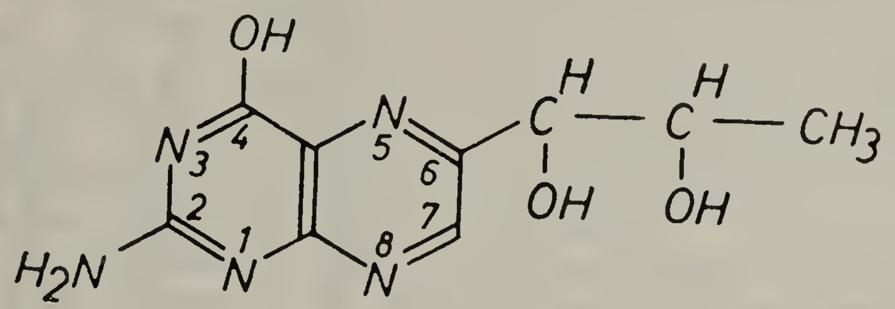
Tab. 2.

Vitamingehalt von Bienenfuttersäften (γ/g Frischsubstanz)

Vitamin:	*Weiselfuttersaft*:	*Arbeiterfuttersaft*:
Thiamin	1,2 - 18	1,2
Riboflavin	6 - 28	10,8
Pyridoxin	2,2 - 50	nicht bestimmt
Nicotinsäure	48 - 125	52
Pantothensäure	110 - 320	26 - 46
Biotin	1,6 - 4,1	2,5 - 3,3
Folsäure	0,16 - 0,5	0,11 - 0,25
Inosit	78 - 150	nicht bestimmt

allem die enormen Stoffwechselleistungen sowohl der heranwachsenden Weisellarve als auch der Bienenkönigin ermöglichen; ein Einfluss auf die Determinierung ist sehr unwahrscheinlich.

Eine weitere Analyse der wasserlöslichen, niedermolekularen Bestandteile des Weiselfutters führte zur Isolierung und Identifizierung des 2-Amino-4-hydroxy-6(L-*erythro*-1'.2'-dihydroxypropyl)-pteridins (6):



Dieses sog. Biopterin, ein im U.V.-Licht hellblau fluoreszierendes Pteridinderivat, ist charakteristisch für den Weiselzellenfuttersaft: es kommt nur im Futter und in den Larven der Königinnen in merklicher Menge vor. Eine quantitative mikrobiologische Bestimmung mit Hilfe der Flagellate *Crithidia fasciculata* ergibt einen Gehalt von 300 γ/g Weiselfutter gegenüber einem Biopteringehalt von nur 20-30 γ im Futtersaft der Arbeiterinnenlarven (7). Ob diese Substanz an der Determinierung beteiligt ist, vermögen wir noch nicht zu sagen. Sie wird in der Larve nicht abgebaut und bleibt bis zur Imago unverändert erhalten, wie wir durch Untersuchungen mit radioaktiv markiertem Biopterin zeigen konnten (8).

Zur weiteren Charakterisierung haben wir die wasserlösliche Fraktion von je 10 Gramm Weisel- und Arbeiterinnenfuttersaft an Dowex 1×8 im Ameisensäuresystem von Potter u. Mitarbb. (9) aufgetrennt und dabei die bei 260 mμ absorbierenden Substanzen bestimmt. Auf diesem Wege müssen sich neben Purinen und Pyrimidinen noch eine Reihe von anderen Verbindungen nachweisen lassen, deren Vergleich eine Aussage über die Beziehungen zwischen den beiden Futtersäften erlaubt.

Die Analyse (vgl. Abb. 1) führte zu dem überraschenden Ergebnis, dass sich die beiden Futter in ihrem Gehalt an den untersuchten Verbindungen weder qualitativ noch quantitativ merklich unterscheiden!

Jede der Hauptfraktionen wurde präparativ papierchromatographisch mit Ammonbikarbonatpuffer (10) weiter aufgetrennt und verglichen. Auf diese Weise haben wir u. a. Adenin und Adenosin (II), Guanosin (IV) und AMP (V) isoliert und identifiziert und bei insgesamt 15 Verbindungen keine Unterschiede zwischen den beiden Futtersäften feststellen können. Die bei der Chromatographie des Weiselzellenfuttersaftes zwischen Fraktion VI und VII erkennbare schwache Absorption rührt von 2-Amino-4-hydroxy-pteridincarbonsäure-(6) her, die aus einer geringen Zersetzung des Biopterins stammen dürfte.

Das Ergebnis unserer bisherigen Untersuchungen lässt sich folgendermassen zusammenfassen:

Der Weiselzellenfuttersaft entspricht in seiner Zusammensetzung weitgehend dem Futtersaft der Arbeiterinnenlarven, als einzige Unterschiede kennen wir bis jetzt einen erhöhten Gehalt an Pantothensäure, Folsäure und Biopterin.

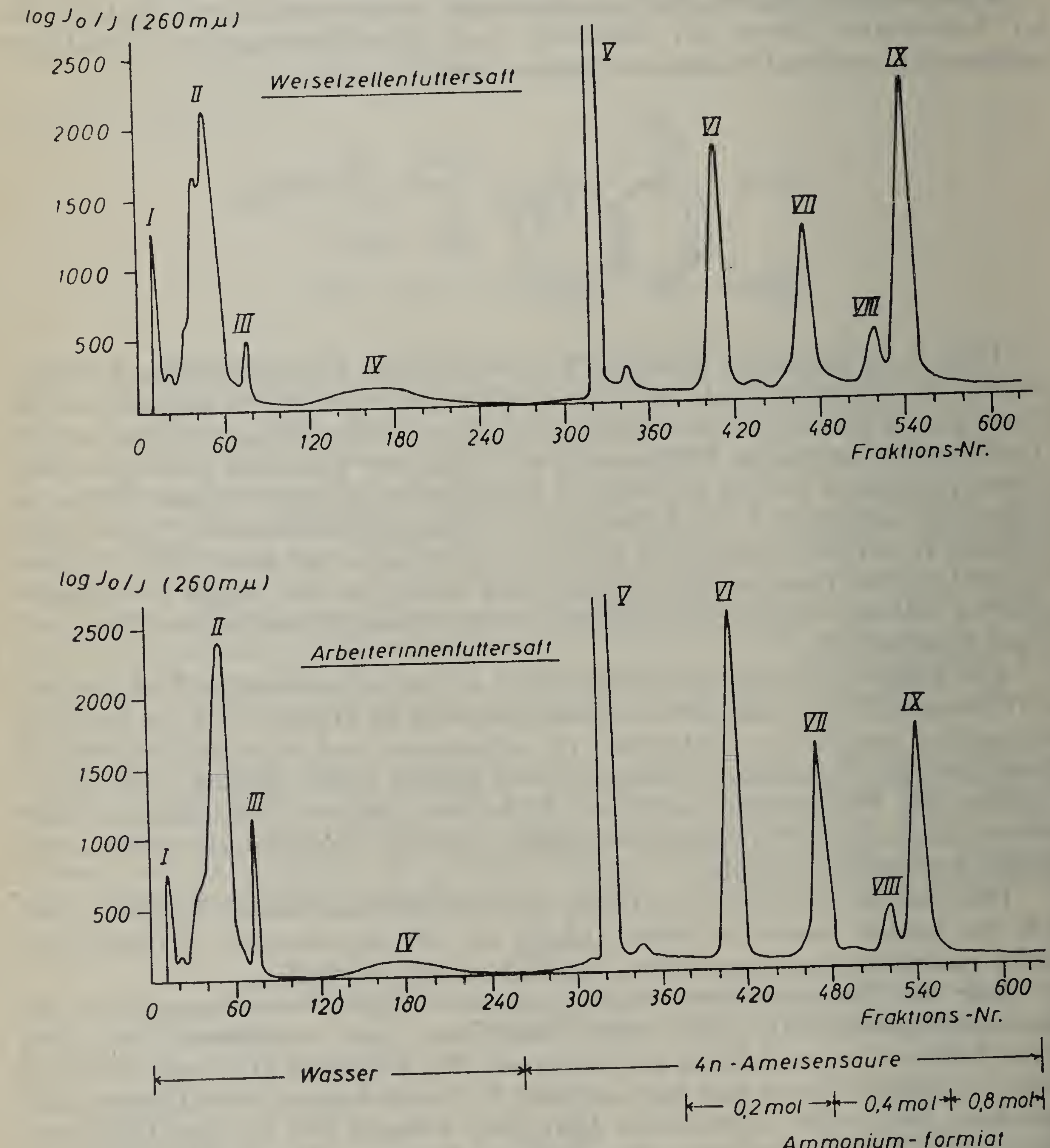


Abb. 1.

Chromatographie des Dialysats von je 10 Gramm Weiselzellen- und Arbeiterinnenfuttersaft an Dowex 1×8 (Formiatform). Fraktionen von je 5 ccm. Ab Fraktion 250 Gradientenelution, Mischgefäss 500 ccm Wasser (9).

Daraus kann man schliessen, dass der Arbeiterinnenfuttersaft das Sekret der Pharyngealdrüse ist und die Grundnahrung aller Bienenlarven bildet, eine Annahme, die auch durch die Ergebnisse von Haydak et al. (11) gestützt wird. Die Autoren berichteten vor kurzem über eine Untersuchung der Proteinkomponenten von Weiselzellen- und Arbeiterinnenfuttersaft, bei der sie ebenfalls keinerlei Unterschiede zwischen den beiden Futtern gefunden haben und die charakteristischen Futtersaftproteine ausschliesslich in der Pharyngealdrüse nachweisen konnten. An der Bildung des Weiselzellenfuttersaftes müssten dann neben der Pharyngealdrüse auch die andern Kopfdrüsen der Ammenbiene beteiligt sein, welche die für diesen Futtersaft charakteristischen Bestandteile dem Pharyngealdrüsensekret zumischen. Untersuchungen, die der Klärung dieser Vorstellung dienen sollen, haben wir begonnen.

Herrn Professor A. Butenandt bin ich für die grosszügige Förderung dieser Arbeiten dankbar. Herrn Dr. K. A. Forster, Fa. H. Mack Nachf., Illertissen, danke ich für die Überlassung der Bienenfuttersäfte, Fräulein S. Schär für ihre technische Unterstützung.

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ZUSAMMENFASSUNG

Das Dialysat von je 10 g Weiselzellen- und Arbeiterinnenfuttersaft wurde an einem Anionenaustauscher (Dowex 1) fraktioniert und sein Gehalt an bei 260 mμ absorbierenden Substanzen bestimmt. Die beiden Futtersäfte unterscheiden sich in der Zusammensetzung der untersuchten Verbindungen nicht merklich. Auf Grund dieses Befundes sowie unter Berücksichtigung früherer analytischer Untersuchungen wird die Bildung des Weiselzellen- und des Arbeiterinnenfuttersaftes aus dem Zusammenwirken der verschiedenen Kopfdrüsen bei der Ammenbiene erklärt.

RIASSUNTO

Contributo alla conoscenza della gelatina reale delle Api.

Dializzati di gelatina reale e di nutrimento per operaie (ambedue in dose di 10 g) vennero frazionati con uno scambiatore di anioni (Dowex 1) e si determinò il loro contenuto in sostanze con assorbimento di 260 mμ. I due prodotti non mostrano sensibili differenze di composizione per quanto riguarda i composti esaminati. In base a questo risultato e tenuto conto di precedenti ricerche analitiche si spiega la formazione della gelatina reale e del nutrimento per operaie con la collaborazione delle diverse glandole cefaliche della nutrice.

Barbier M. (*)

RECHERCHES SUR LA FRACTION ATTRACTIVE DES REINES D'ABEILLES (*APIS MELLIFICA* L.)

La sécrétion des glandes mandibulaires de reines d'abeilles possède trois activités biologiquement connues:

— L'attractivité vis à vis des jeunes abeilles ouvrières (1, 2);

— L'inhibition de la construction des cellules royales (3, 4);

— L'inhibition du développement des ovaires des ouvrières (3, 5).

Au cours des quatre dernières années, nous avons cherché à isoler et à identifier les substances responsables de ces activités. Nous exposerons dans ce qui suit les résultats obtenus lors des divers fractionnements. Les essais biologiques ont été réalisés par Mlle J. Pain (16).

Les reines d'abeilles broyées ont été extraites soit par l'éthanol, soit par le butanol tertiaire. Les extraits bruts ont été séparés en fractions solubles et insolubles dans l'acétone; puis la fraction soluble dans l'acétone, mise en suspension dans un peu d'eau à pH 2 est extraite successivement par le pentane, l'éther et le chloroforme; chaque phase organique est ensuite séparée en acides et en neutres par le carbonate de sodium 2 N. [Pour la technique exacte de ces séparations voir (6)]. Les fractions acides extraites du pentane contiennent à la fois les trois activités.

A partir des fractions neutres extraites du pentane, nous avons isolé et identifié (6, 7) le 24 méthylène-cholestérol. Nous avons montré plus tard (8) que ce stérol n'était pas un produit du métabolisme des abeilles; nous l'avons en effet isolé en quantités importantes de diverses espèces de pollen.

Les fractions neutres étant dépourvues d'activité, les recherches ont été poursuivies sur les parties acides obtenues du pentane. Ces acides ont été distillés. La partie distillant de 20 à 90° sous 0,05 mm est active. 0,1 mg déposés sur un papier filtre attirent plus de 10 abeilles. 5 mg suffisent à produire une inhibition de 80 % du développement des ovaires de 15 abeilles.

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Les séparations que nous venons de décrire, ont été répétées avec 1 Kg d'abeilles ouvrières d'une part, et 1 Kg d'abeilles orphelines d'autre part. Les acides obtenus à partir des abeilles ouvrières sont faiblement attractifs; la quantité totale correspond à celle que l'on pourrait obtenir à partir de 14 reines. La fraction acide obtenue des abeilles orphelines est absolument inatractive (9).

L'examen de la fraction active isolée des reines, a été effectué par chromatographie sur colonne d'acide silicique, ou sur papier. Ces techniques ont toujours conduit à une perte totale de l'attractivité. Cette attractivité peut être reconstituée en mélangeant les éluats benzèniques et éthérés de la chromatographie sur colonne, ou les éluats des zônes de R_F compris entre 0,5 et 1 de la chromatographie sur papier (système chloroforme-formamide). L'attractivité est donc dûe à un mélange de substances acides (9).

Par chromatographie préparative sur papier dans le système cité, nous avons pu isoler trois substances à l'état cristallisé (10).

Une substance phénolique F. 128° R_F 0,5 a été identifiée au p-hydroxybenzoate de méthyle; nous n'avons pas encore réussi à prouver que cette substance était introduite dans la nourriture d'appoint donnée aux abeilles.

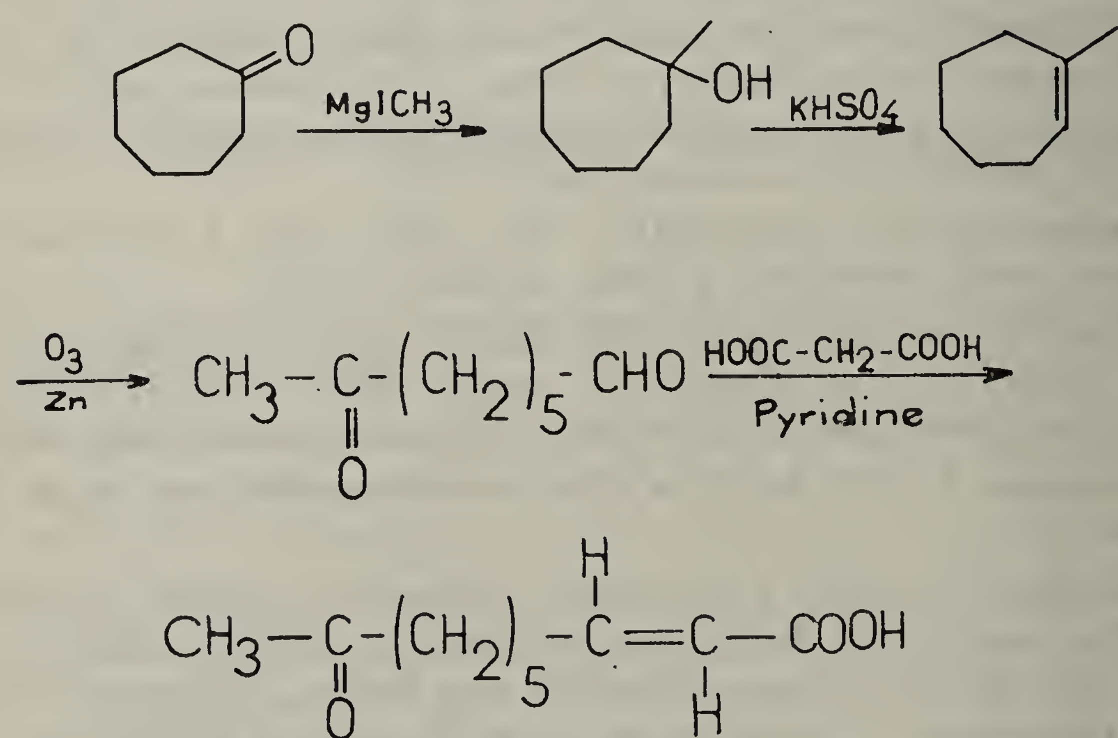
Une substance F. 93° R_F 1, n'a pu être identifiée, par suite du peu de produit isolé.

Un acide F. 55° R_F 0,85 a également été isolé; cet acide est identique par ses propriétés inhibitrices de la construction des cellules royales, à un acide F. 50° isolé des têtes de reines d'abeilles par Butler, Callow et Johnston (4). Nous avons déterminé la structure de cet acide grâce à l'utilisation d'un chromatographe à détecteur ionique Argon Pye, chaque réaction étant effectuée sur des quantités de substance de l'ordre de 0,3 mg. Les résultats obtenus, ainsi que les spectres UV et IR sont en accord avec la structure d'un acide céto-9 décène-2 oïque (11). Callow et Johnston (12) sont arrivés à la même conclusion quant à la structure de cette substance. Les deux travaux ont été publiés à une semaine d'intervalle. Callow et Johnston ont en outre décrit une synthèse de cet acide, en 9 étapes, à partir de l'acide azélaïque. Ils obtiennent un acide F. 45-52° biologiquement actif. Nous avions de notre part entrepris cette synthèse à partir de la cycloheptanone; en quatre étapes, et avec un rendement global de 20 %, nous avons obtenu un acide céto-9 décène-2 oïque F. 51-54° biologiquement actif (13). 0,05 mg suffisent à inhiber la construction des cellulles royales dans une population de 150 abeilles. Par une méthode analogue, nous avons synthétisé l'acide céto-8 nonène-2 oïque F. 48-51°, qui à la concentration de 0,120 mg pour 150 abeilles est inactif. Nous attribuons à l'acide céto-9 décène-2 oïque naturel, la configuration *trans*; la présence d'une bande à 10,12 μ dans le spectre IR, ainsi que l'identité du produit naturel avec le produit obtenu par condensation selon Knoevenagel sont en faveur de cette hypothèse.

L'acide inhibiteur de la construction des cellules royales est inattractif, mais il est indispensable dans le mélange attractif. Si l'on distille de nouveau la fraction active, en recueillant la partie la plus volatile (20 à 60° sous 0,1 mm)

on obtient un mélange inattractif, mais l'adjonction d'acide céto-9 décène-2 oïque reconstitue l'attractivité (2).

Nous avons poursuivi l'analyse de la fraction très volatile du mélange attractif par chromatographie en phase gazeuse. A 150°, les esters méthyliques préparés par action du diazométhane donnent 5 pics. Les deux premiers pics ont des volumes de rétention qui correspondent aux volumes de rétention des phénylacétate et phénylpropionate de méthyle.



Les glandes mandibulaires des reines d'abeilles étant elles-mêmes attractives, nous avons continué ces essais, en injectant directement dans le chromatographe, les extraits éthérés, estérifiés, des glandes mandibulaires (2). Tout d'abord, à 200°, on remarque que le pic principal correspond à l'ester méthylique de l'acide céto-9 décène-2 oïque; il existe aussi trois autres pics de très faible intensité, dont les volumes de rétention sont identiques à ceux du p-hydroxy-benzoate de méthyle, de l'azélaate de méthyle, et du sébaçate de méthyle. Nous avons vérifié la présence du p-hydroxybenzoate en injectant dans les mêmes conditions le mélange non estérifié; le seul pic observé est celui dont le volume de rétention correspond au p-hydroxy-benzoate de méthyle. La chromatographie sur papier des extraits de glandes mandibulaires montre également une tache de même R_F que la substance témoin (conditions déjà citées). Si l'on abandonne les extraits de glandes avec un excès de diazométhane pendant plusieurs heures, on remarque la formation d'une odeur d'anisate de méthyle. Un travail parallèle effectué sur les glandes mandibulaires des abeilles ouvrières, n'a pas permis de retrouver le p-hydroxy-benzoate de méthyle. Le principal acide présent est alors l'acide hydroxy-10 décène-2 oïque; il existe en outre de faibles quantités d'un acide, dont l'ester méthylique possède le même volume de rétention que le sébaçate de méthyle. A 150°, les esters méthyliques préparés

à partir des extraits de glandes mandibulaires de reines montrent 5 pics, dont les volumes de rétention sont identiques à ceux des 5 premiers pics observés lors de l'examen effectué sur les esters des extraits totaux de reines. Les 4 premiers pics sont d'intensité très faible; le 5ème est plus important. Nous supposons que la substance représentée par ce 5ème pic joue un rôle prépondérant dans l'odeur attractive, de même que l'acide céto-9 décène-2 oïque.

Une étude systématique a alors été réalisée, sur les sécrétions mandibulaires des différentes catégories de reines (reines naissantes, vierges âgées, reines fécondes, reines bourdonneuses...). En effet, des différences d'activité ont été remarquées en fonction de ces différences (14). L'étude des esters méthyliques dans le gaz-chromatographe, à 200°, montre que dans tous les cas, l'acide céto-9 décène-2 oïque est présent, mais en quantités plus ou moins importantes, le facteur déterminant étant l'âge. Plus une reine est âgée, quelque soit son état, et plus elle sécrète d'acide inhibiteur de la construction des cellules royales (15). L'étude effectué à 150°, montre de même, que les 5 pics présents varient en importance avec l'âge; ils sont indécelables chez les reines naissantes et très jeunes; il est remarquable de constater que ces reines sont inattractives.

Nous continuons les recherches en vue de l'identification de toutes les substances présentes dans le mélange attractif, ainsi que de la substance responsable de l'inhibition ovarienne.

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RÉSUMÉ

La sécrétion des glandes mandibulaires des reines d'abeilles, possède trois activités biologiques connues. Nous avons cherché à isoler et identifier les substances responsables de ces activités, et décrivons les résultats obtenus au cours des dernières années.

L'attractivité vis-à-vis des jeunes ouvrières paraît être dûe à un mélange de substances acides volatiles, dont l'étude chromatographique à été faite. L'inhibition de la construction des cellules royales, est dûe à l'acide céto-9 décène-2 trans oïque, que nous avons isolé, identifié, et synthétisé. La substance responsable de l'inhibition du développement des ovaires des ouvrières demeure inconnue.

RIASSUNTO

Ricerche sulla frazione attrattiva delle regine di Ape (Apis mellifica *L.*).

La secrezione delle glandole mandibolari delle regine di ape ha tre attività biologiche conosciute. Abbiamo cercato di isolare e d'identificare le sostanze responsabili di tali attività, e descriviamo i risultati ottenuti negli ultimi anni.

L'attrattività esercitata sulle giovani operaie sembra dovuta ad una mescolanza di sostanze acide volatili delle quali è stato fatto lo studio cromatografico.

L'inibizione della costruzione delle cellule reali è dovuta all'acido cheto-9-decen-2-trans-oico che abbiamo isolato, identificato e sintetizzato.

La sostanza responsabile dell'inibizione dello sviluppo ovarico delle operaie non è conosciuta.

WECKERING R. (*)

I. - STEREOELECTRONIE DE DIVERS COMPOSES EXISTANT DANS LES CORPS D'INSECTES

Pour l'étude des phénomènes biologiques il est de toute première importance qu'on soit exactement renseigné sur les structures moléculaires stériques intégrales des composés intervenant dans les phénomènes.

La « *Théorie de l'atome à champ nodique* » ou « *Mécanique Ondulatoire Stérique* » permet d'établir les structures moléculaires stéréonucléaires et stéréoélectroniques, avec indication des dimensions approximatives en valeurs absolues, de pratiquement tous les composés chimiques.

Avant d'indiquer les principes fondamentaux de la théorie atomique nodique, nous en donnons quelques exemples d'application, en choisissant des molécules de composés existant dans les corps d'insectes.

1. LEUCOPTÉRINE

Les ptérines, entre autres la leucoptérine, existent dans les ailes de papillons et d'autres insectes. Structure schématique de la leucoptérine, fig. 2. La fig. 3

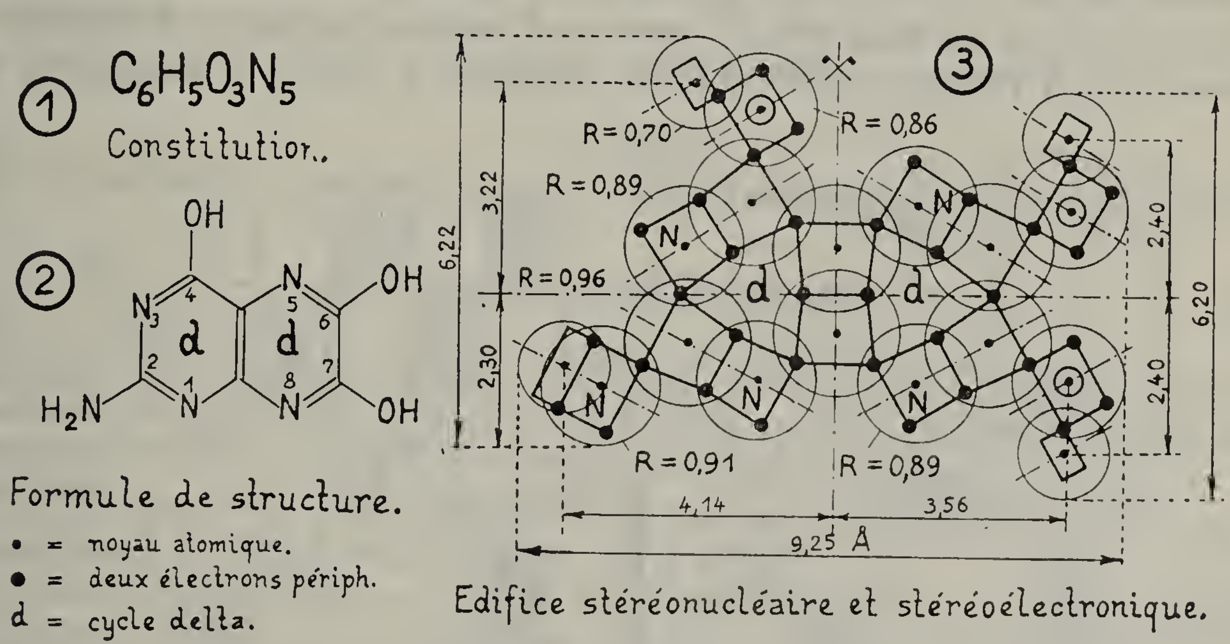

Fig. 1 - 3.
Leucoptérine. - Echelle de la fig. 3: 1 cm = 1,92 A.

(*) *Ingénieur. Beho (Gouvy), Belgique.*

en représente *la structure stéréonucléaire* (la constellation spaciale des noyaux atomiques) et en même temps *la structure stéréoélectronique* (la constellation spaciale des électrons) à l'échelle, selon notre théorie, avec indication de quelques dimensions principales (approximation estimée à ± 5 %).

La molécule est plane, abstraction faite des deux atomes d'hydrogène du groupe NH_2, qui se trouvent l'un à l'avant, l'autre à l'arrière du plan de la molécule. (Il est connu que la molécule de NH_3 n'est pas plane).

2. PÉRIPLANÉTINE

La périplanétine fut isolée de la sécrétion de l'organe latérocervical de *Periplaneta americana L.* et de *Blatta orientalis L.* par M. Pavan en 1954; l'étude de sa structure chimique fut entreprise en 1958 par l'équipe Quilico, Piozzi, Pavan et Mantica. Ces recherches conduisaient à la constitution 1-benzoil-β-d-glucose, fig. 11, qui correspond à celle d'une substance préparée antérieurement (1931) par L. Zervas, mais qu'on n'avait pas encore trouvée dans la nature.

Par hydrolyse de la périplanétine on en sépare la d-glucose. En appliquant à la formule de structure connue de cette dernière (fig. 6) notre méthode du gabarit des polyalcools (voir page 358 de notre ouvrage « The Nodic Field Atom »), on obtient pour la molécule de ce composé la structure stérique fig. 7.

La structure stéréoélectronique de la périplanétine est ainsi donnée par la fig. 12. Les trois radicaux OH rattachés à des chaînons du cycle central, se trouvent d'un même côté du plan du cycle, puisque c'est le radical OH qui dans la d-glucose se trouve *de l'autre côté* du plan de la molécule (celui rattaché à 5), qui ferme par son atome d'O le cycle entre 1 et 5.

Il y a cinq atomes asymétriques (No 1 à 5) dans la périplanétine; mais comme les trois qui portent des H.OH ne forment ensemble qu'un seul centre d'asymétrie, il n'y a en tout que 3 centres d'activité optique. Théoriquement il existe donc $2^3 = 8$ formes moléculaires, formant 4 paires d'antipodes optiques.

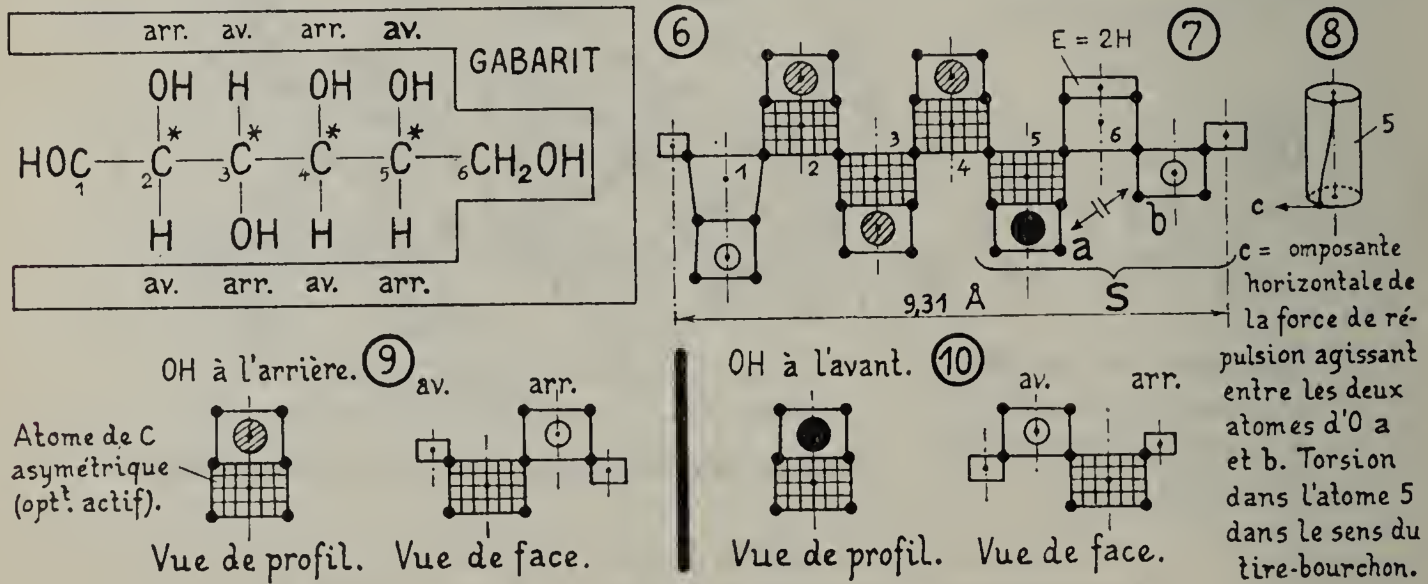

Fig. 6 - 10

d-Glucose. - S = groupe commun à toutes les aldoses dextrogyres.
Echelle des fig. 7, 9 et 10: 1 cm = 2,1 A.

La paire la plus stable seule se forme normalement dans la nature; c'est probablement celle où, par suite d'un effet de cohésion, les trois OH et les deux radicaux lourds C_6H_5CO- et -C_2OH se trouvent tous d'un même côté du plan du cycle central.

La fig. 12 représente l'un des antipodes optiques de la périplanétine; son image dans un miroir en représente l'autre antipode.

Les sens de torsion dans les atomes 1 et 5 sont opposés.

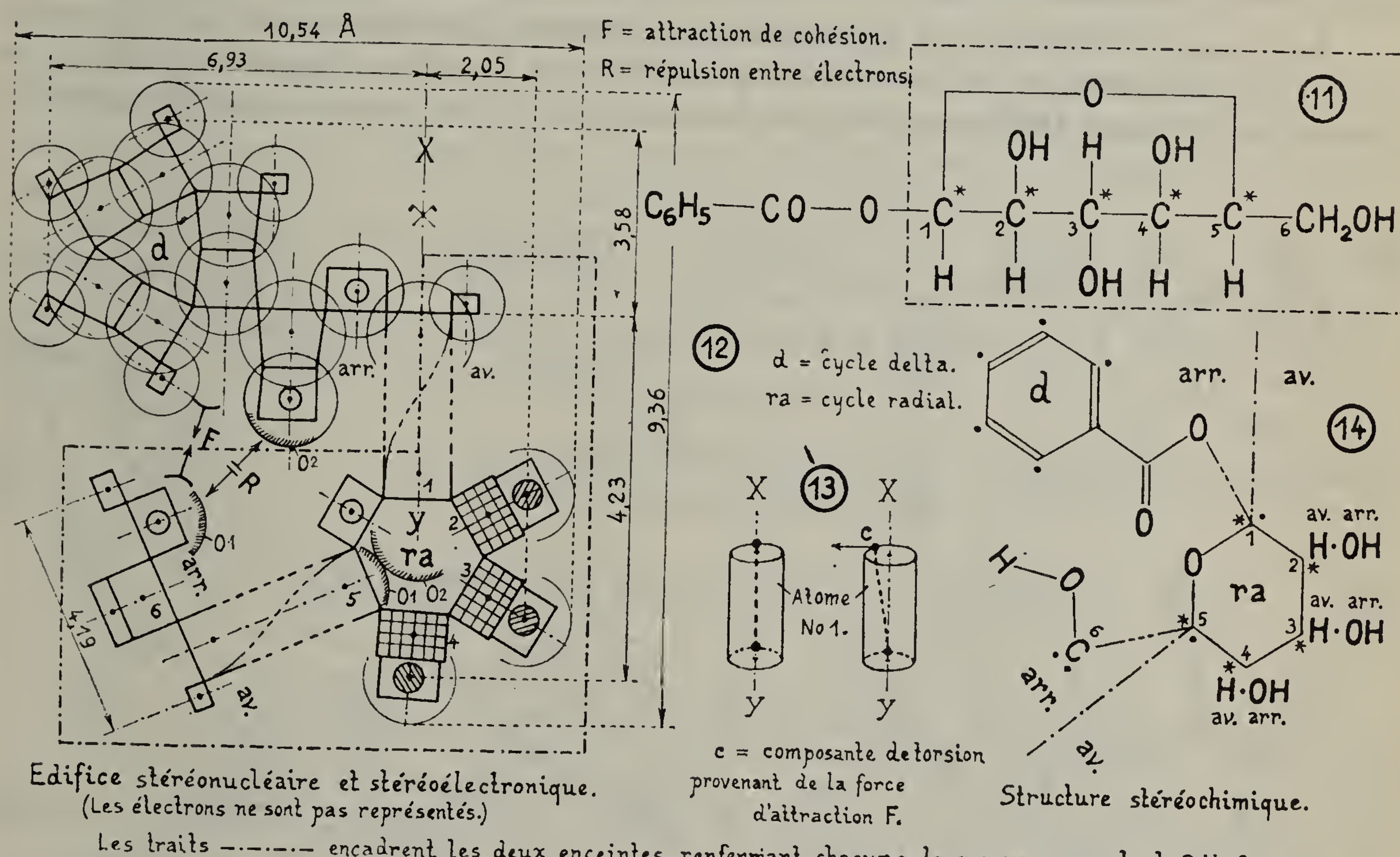

Fig. 11 - 14.

Périplanétine (1-benzoïl-β-d-glucose). - Echelle de la fig. 12: 1 cm = 2,08 A.

3. ACIDE CARMINIQUE

Du corps de la femelle de *Coccus cacti* (cochenille) on extrait l'acide carminique, qui est un des plus beaux et des plus solides colorants pour mordants, mais aussi un des plus chers. Dimroth lui attribue la formule de structure fig. 15.

Dans ce composé il y a le groupe monovalent $C_6H_{11}O_5$, que nous avons rencontré déjà dans la périplanétine (fig. 11 à 14).

Formule de structure complétée, fig. 17. Structure stéréochimique détaillée, fig. 16. Structure stéréonucléaire et stéréoélectronique, fig. 18 et 19.

Il y a dans cette molécule sept centres d'asymétrie:

1. - L'ensemble formé par les 8 atomes asymétriques touchant les deux failles (fig. 17), les trois groupes plans P, R et S (fig. 19) étant disposés en escalier.

2. - L'ensemble formé par les trois cyclons 16, 17 et 18 (caractéristique de la d-glucose); les trois groupes OH se trouvent tous soit à l'avant, soit à l'arrière.

3. à 7. - Les atomes asymétriques 1, 7, 14, 15 et 19.

Ces sept centres d'asymétrie ne font toutefois qu'un seul, si nous acceptons le principe de la formation dans la nature de la seule forme la plus stable, *celle en forme d'escalier d'un bout à l'autre de la molécule*, selon fig. 19.

Le groupe COOH peut cependant se trouver, théoriquement, soit en endo sur 6 (fig. 18), soit en exo sur 6 (en pointillé sur la même figure); il en est de même des groupes OH rattachés aux atomes 2 et 13, et du groupe CH_3 rattaché

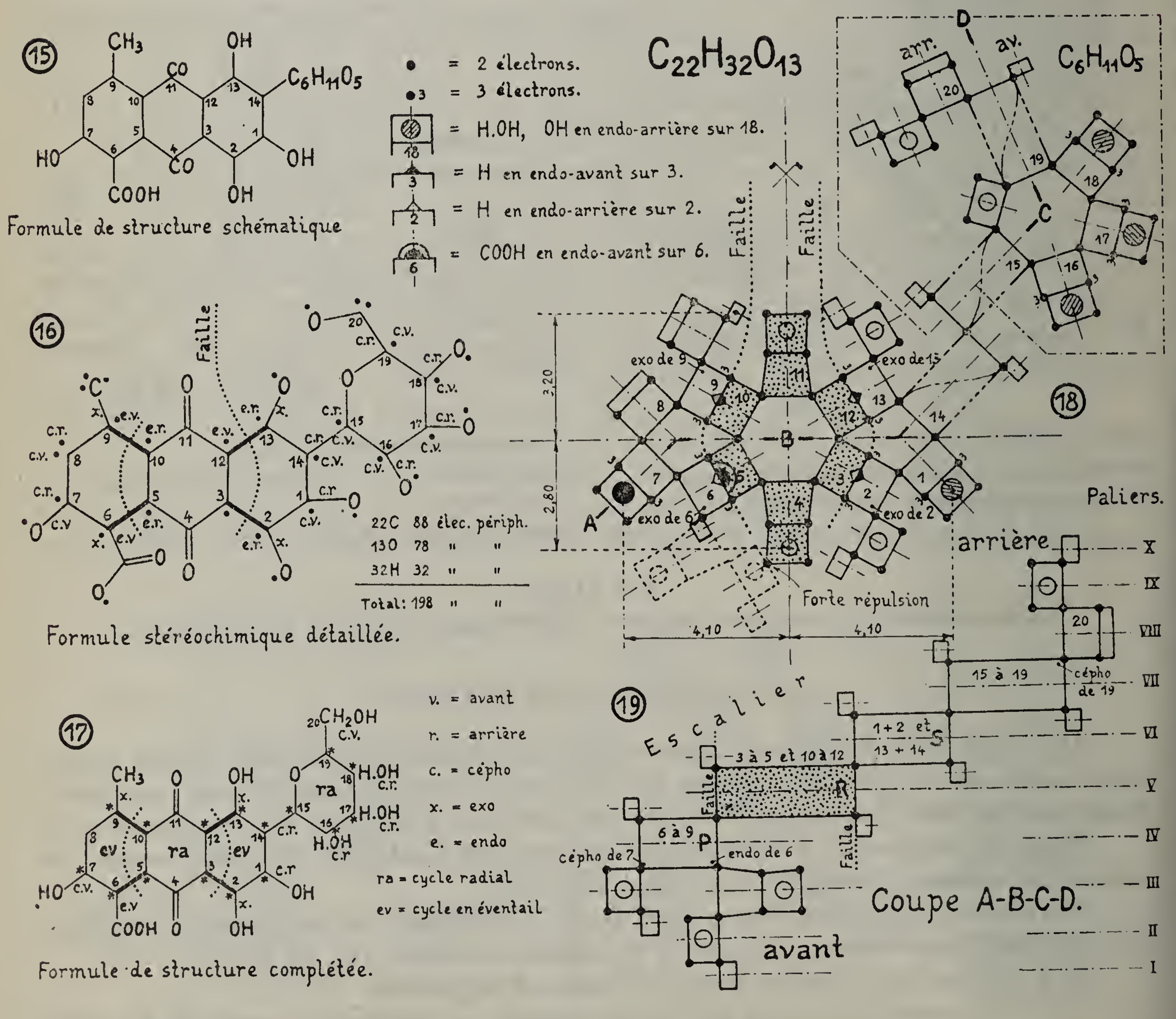

Fig. 15 - 19.

Acide carminique. - Echelle des fig. 18 et 19: 1 cm = 2,22 A.

à l'atome 9. Les emplacements en exo sont normalement les plus stables. Le groupe COOH n'est cependant pas stable en exo par suite d'une gêne stérique (forte répulsion entre deux atomes d'O, voir fig. 18); sa position stable sera donc en endo.

4. RIBOFLAVINE

La vitamine B_2, lactoflavine ou riboflavine, est une vitamine de croissance très répandue dans le règne animal et végétal. Fig. 23.

Structure stéréonucléaire et stéréoélectronique à l'échelle, fig. 24.

Faisons remarquer que le groupe —C = C— et celui de —C = N— insérés dans un cycle sont de dimensions moindres que les mêmes groupes non insérés dans un cycle. (Voir à ce sujet aussi les fig. 3, 31 et 35).

Le plan de la partie hachurée H–2–3–4–5–H est déplacé en arrière (par rapport au plan des deux cycles vo) de l'épaisseur d'un octet électronique dans l'un des antipodes optiques (fig. 24); il est déplacé en avant de la même épaisseur dans l'autre antipode.

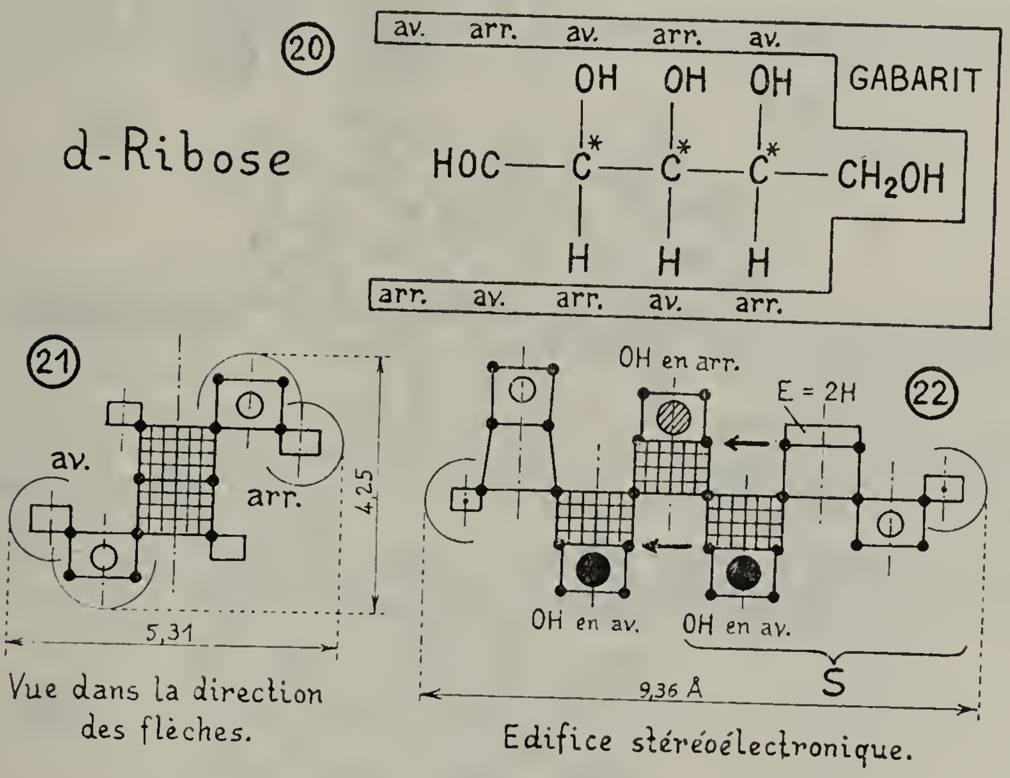

Fig. 20 - 22.
d-Ribose. - S = groupe commun à toutes les aldoses dextrogires.
Echelle des fig. 21 et 22: 1 cm = 2,05 A.

La chaîne latérale provient de la d-ribose; fig. 20 à 22.

La molécule de riboflavine possède non pas 8 (fig. 23), mais 11 atomes asymétriques (fig. 25); elle ne possède cependant que trois centres d'activité optique:

1. - L'ensemble des trois atomes de carbone 16, 17 et 18.

2. - L'ensemble des quatre atomes asymétriques touchant la faille et des atomes de carbone 3 et 4 (disposition en escalier).

3. - L'atome d'azote 14.

Nombre des formes moléculaires: $2^3 = 8$; il y a donc quatre paires d'antipodes optiques.

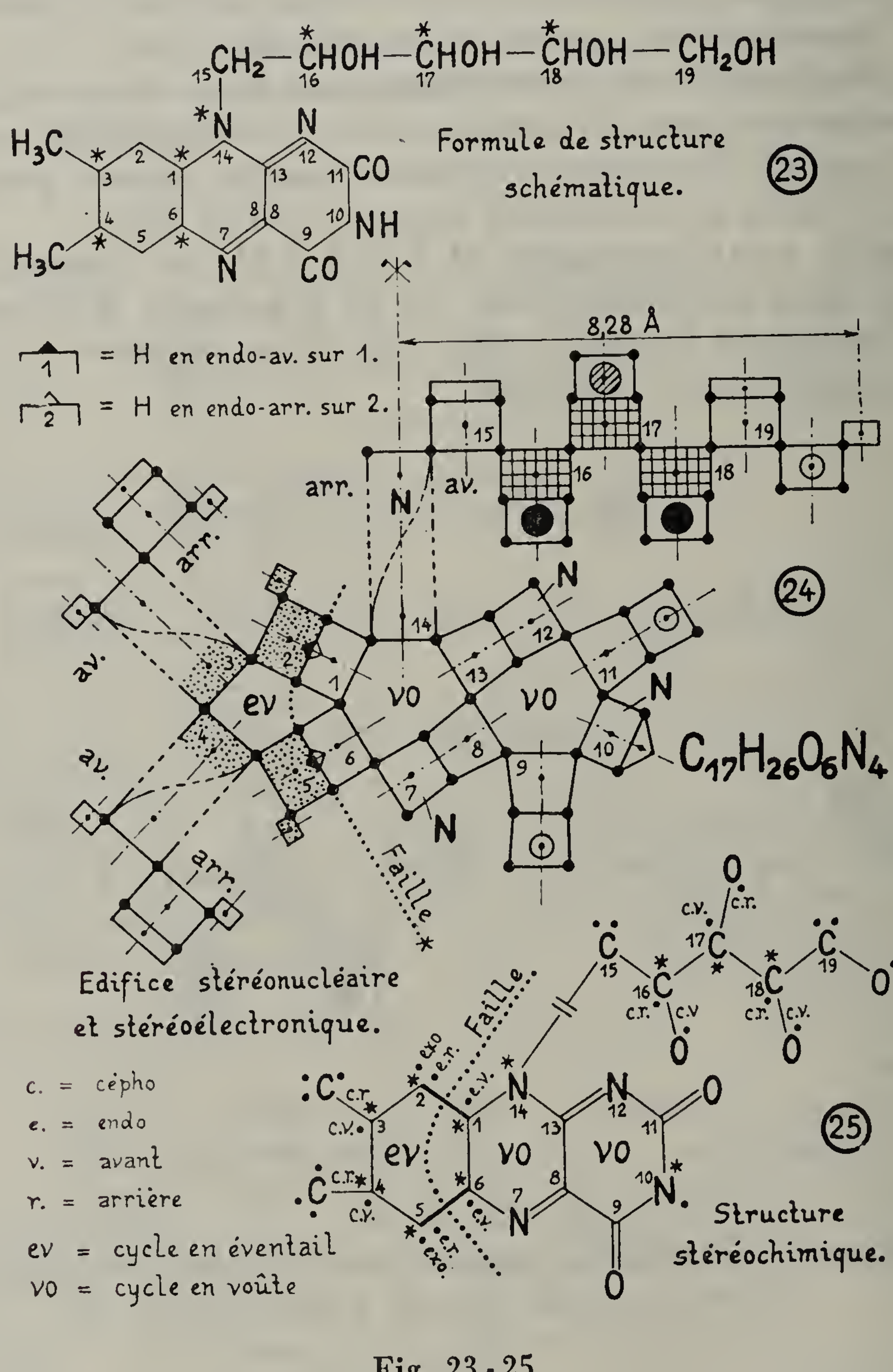

Fig. 23 - 25.

Riboflavine. - Echelle de la fig. 24: 1 cm = 2,1 A.

Bien que l'atome d'azote 10 soit asymétrique, il n'augmente pas du fait de sa présence le nombre des paires d'antipodes optiques de la riboflavine. En effet, l'atome d'hydrogène rattaché à lui se déplace avec grande facilité de l'arête-

avant à l'arête-arrière de l'atome d'azote et vis-versa; il adoptera donc dans chaque forme moléculaire optiquement active la seule position la plus stable. Relevons qu'on ignore en chimie doctrinale la cause de la non-activité optique apparente de l'atome d'azote asymétrique.

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RÉSUMÉ

Pour une étude fructueuse des phénomènes biologiques il est de toute importance qu'on soit exactement renseigné sur les structures moléculaires stériques intégrales des composés intervenant dans les phénomènes.

La « Théorie de l'atome stérique à champ nodique », ou « Mécanique Ondulatoire Géométrique », élaborée par l'auteur, permet d'établir les structures moléculaires stéréoélectroniques, avec indication des dimensions en valeurs absolues, de pratiquement tous les composés chimiques.

Sont décrites en détails les structures stéréoélectroniques des molécules des composés suivants, existant dans les corps d'insectes: leucoptérine, périplanétine, acide carminique, riboflavine.

RIASSUNTO

I. - Stereoelettronica di diversi composti esistenti nei corpi di Insetti.

Per uno studio fruttuoso dei fenomeni biologici è di importanza primaria essere esattamente informati sulle strutture molecolari steriche integrali dei composti che prendono parte ai fenomeni.

La « Teoria dell'atomo sterico a campo nodico » o « Meccanica ondulatoria geometrica », elaborata dall'autore, permette di stabilire la struttura molecolare stereoelettronica con le indicazioni in valore assoluto praticamente di tutti i composti chimici.

Sono descritte nei particolari le strutture stereoelettroniche delle molecole dei composti seguenti esistenti nei corpi di Insetti: leucopterina, periplanetina, acido carminico, riboflavina.

WECKERING R. (*)

II. - STEREOELECTRONIE DE POISONS D'ABEILLES, DE GUEPES ET DE FRELONS

Pour la compréhension du bien-fondé de nos représentations intégrales dans l'espace à trois dimensions des édifices moléculaires, il est indiqué que nous donnions un court résumé de la *théorie de l'atome à champ nodique* ou *Mécanique Ondulatoire Stérique.*

A chaque enveloppe atomique est associé, selon cette théorie, un champ électro-magnétique à ondes stationnaires ou *champ nodique*, possédant des noeuds électriques (à ventres magnétiques) qui sont situés sur des niveaux sphériques concentriques successifs K, L, M, N, O, ... Le noyau positif de l'atome se trouve au centre commun de ces niveaux. Le nombre de noeuds par niveau et le mode de leur distribution sur les niveaux ressortent clairement des fig. 26 et 27.

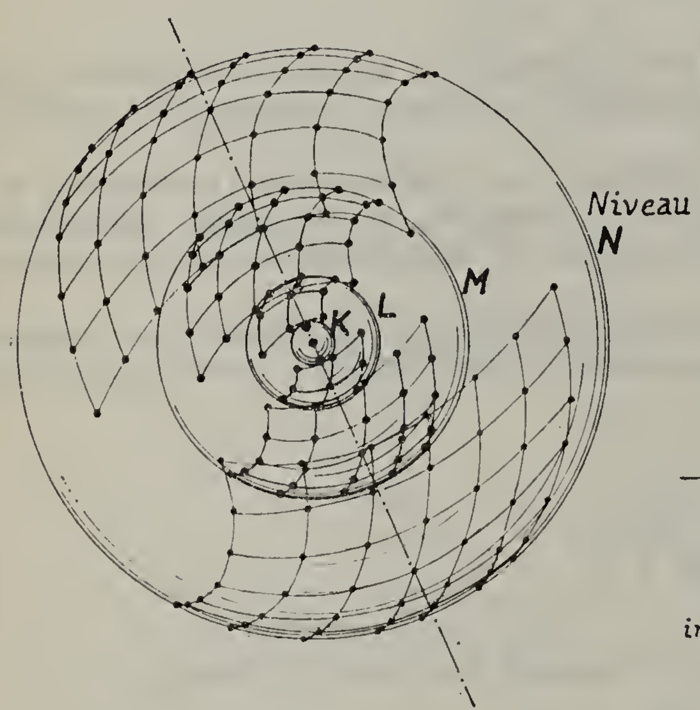

Fig. 26.
Vue en perspective de la constellation des noeuds du champ électro-magnétique nodique de structure unique et universel.

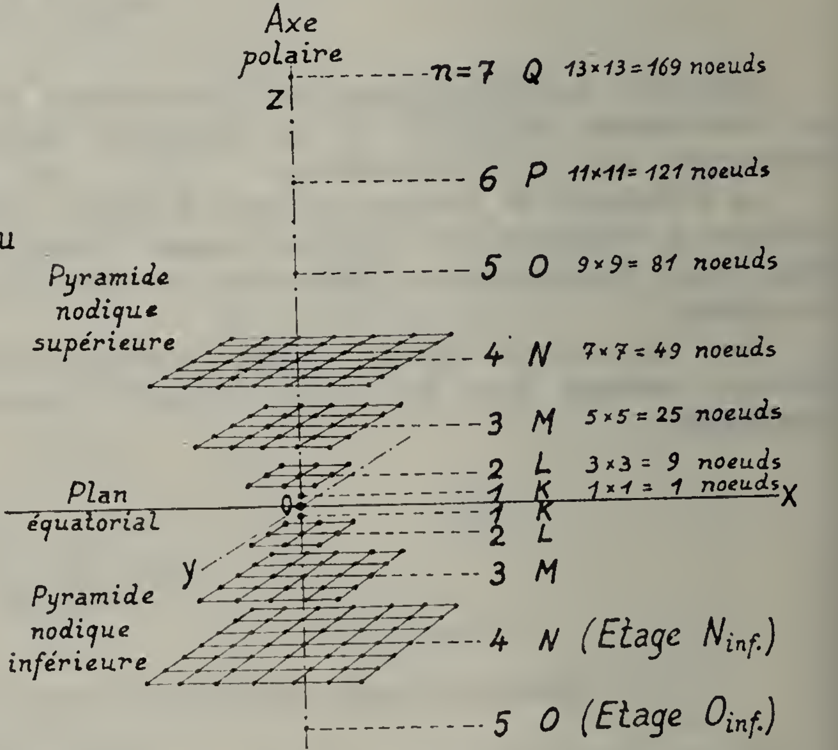

Fig. 27.
Représentation «schématique» du champ nodique.

Uniquement les noeuds électriques sont représentés sur ces deux figures.

(*) *Ingénieur. Beho (Gouvy), Belgique.*

Les électrons d'un atome sont fixés élastiquement à certains noeuds électriques du champ nodique. Ce dernier possède un axe de symétrie privilégié, appelé *axe polaire*, que nous plaçons toujours verticalement, et un plan de symétrie privilégié perpendiculaire à cet axe, appelé *plan équatorial*. Ce plan subdivise chaque niveau nodique sphérique en deux demi-niveaux, un supérieur et un inférieur. Il est commode de «schématiser» le réseau nodique afin de simplifier les figures, en représentant les demi-niveaux convexes par des plans parallèles au plan équatorial, et les noeuds sur ces plans à des distances égales les uns des autres, comme le fait voir la fig. 27.

Chaque demi-niveau d'un atome muni d'électrons s'appelle *étage*, et les deux étages périphériques de l'atome s'appellent *tables*. Chaque atome possède donc une table supérieure et une table inférieure. A ces tables sont liées les propriétés chimiques et cristallines des atomes des divers éléments.

Sur un même étage schématisé dans un plan, chaque deuxième noeud seulement en les directions orthogonales et obliques à 45° peut être occupé par un électron; ces noeuds, en effet, se trouvent en une même phase de vibration magnétique.

Sur chaque étage intérieur d'un atome, les électrons forment des configurations simples de grande stabilité, symétriques par rapport à l'axe polaire, à 1,

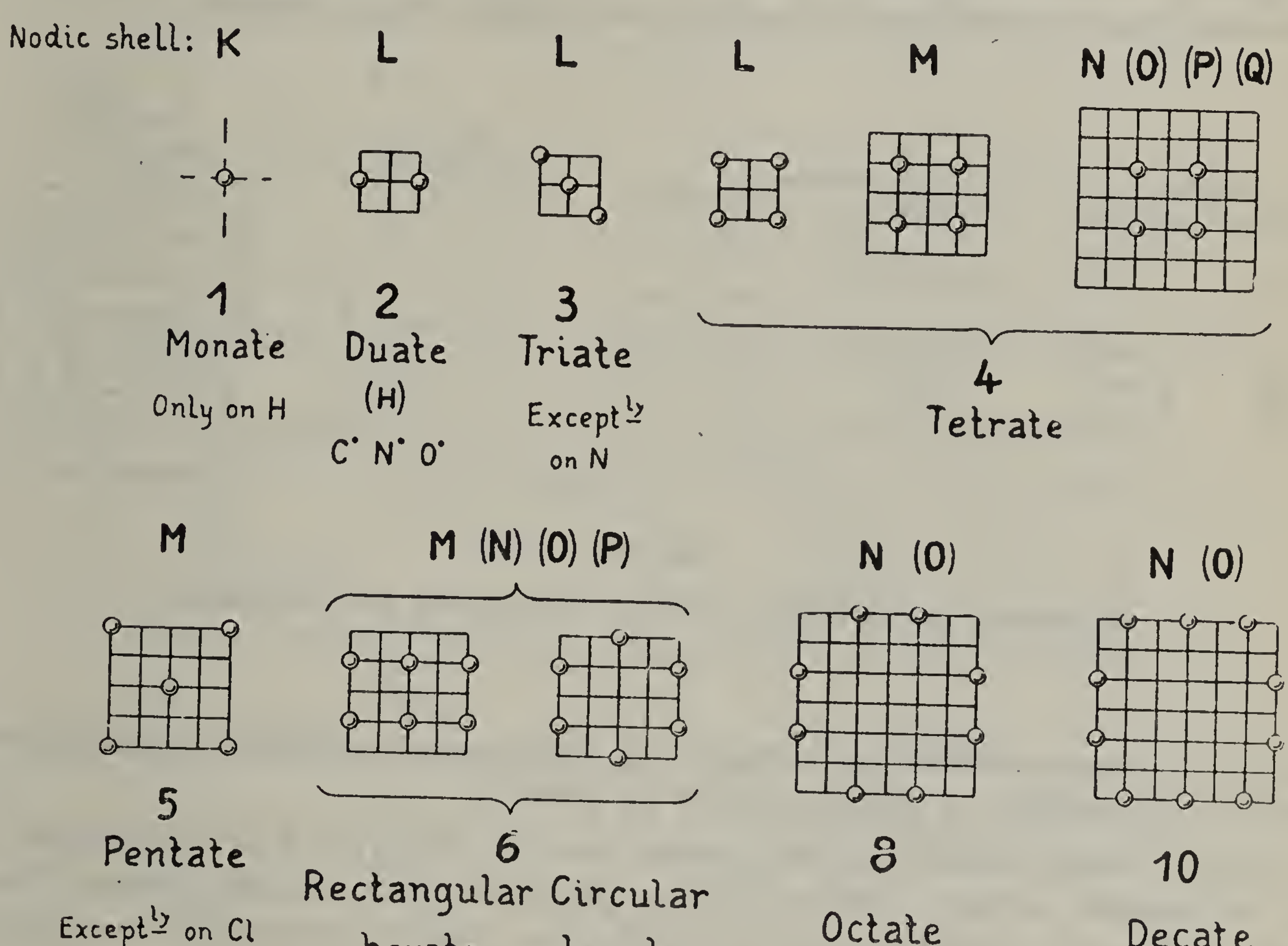

Fig. 28.

Electronats fermés de très grande stabilité (schématisés dans un plan).

4, 9 et 16 particules, appelées *électronats fermés*, fig. 28. Sur cette base il nous a été facile d'établir les structures stériques complètes des enveloppes atomiques de tous les éléments.

L'existence dans chaque atome d'un axe privilégié, l'axe polaire, reconnue expérimentalement par J. Stark déjà en 1927, est un fait d'une importance capitale, non pris en considération en physique atomique et stéréochimie doctrinales.

Dans la moitié supérieure et la moitié inférieure de tout atome, les axes de spin des électrons sont synoparallèles respectivement antiparallèles à l'axe polaire considéré comme un vecteur, de sorte que le spin résultant de l'atome est nul chaque fois que les deux moitiés contiennent le même nombre des électrons.

Aux tables des atomes insérés dans des molécules ou des cristaux, on trouve des *électronats périphériques stables* à 0, 1, 4, 6, 8 et 10 électrons, symétriques par rapport aux axes polaires, fig. 29.

A leur état isolé, les atomes de tous les éléments, exceptés ceux des gaz nobles, ne possèdent pas assez d'électrons à leur niveau périphérique pour y parfaire deux électronats complets. Ces atomes cherchent à se réunir de manière que chacun d'eux soit muni de deux électronats périphériques stables; ainsi s'établissent tout naturellement les liaisons stables entre atomes.

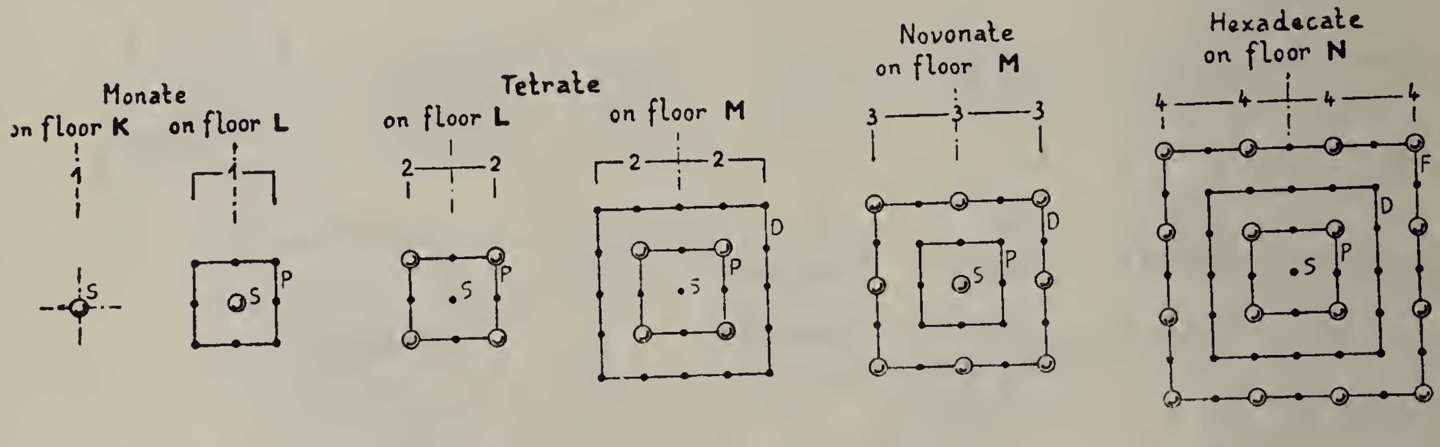

Fig. 29.

Electronats périphériques stables (schématisés dans un plan).

Principe fondamental concernant les structures atomiques, moléculaires et cristallines: *Symétrie et stabilité vont de pair.*

Chaque noeud normalement occupé par un électron d'un électronat déterminé, est appelé *cellule*. Deux cellules périphériques voisines, dont l'une est occupée et l'autre vide, forment ensemble une valence chimique ou cristalline. Deux cellules occupées communes à deux atomes, forment une *liaison* chimique ou cristalline. Dans les cristaux existe aussi la semi-liaison, formée par une seule cellule occupée commune aux deux atomes. La tendance au parallélisme des

axes de spin des électrons détermine la direction des valences et des liaisons. Pour l'origine de cette direction, on ne connaît pas d'explication en chimie-physique doctrinale.

La tendance à la disposition symétrique des atomes rattachés à un atome déterminé par rapport à l'axe polaire de ce dernier, explique une multitude de constatations expérimentales en stéréochimie, restées inexpliquées en science doctrinale. Exemples: forme angulaire de H_2O; forme en zigzag de la chaîne carbonique normale.

Contrairement aux opinions courantes, la structure de la maille cristalline élémentaire d'une substance est t o u j o u r s d i f f é r e n t e de celle de sa molécule.

Le saut d'un électron d'un noeud à un autre ou jusqu'en dehors de la région propre d'un atome, ou le passage d'un état de résonance à un autre, correspond à l'émission ou à l'absorption d'un photon quantique électro-magnétique, donc à une raie spectrale.

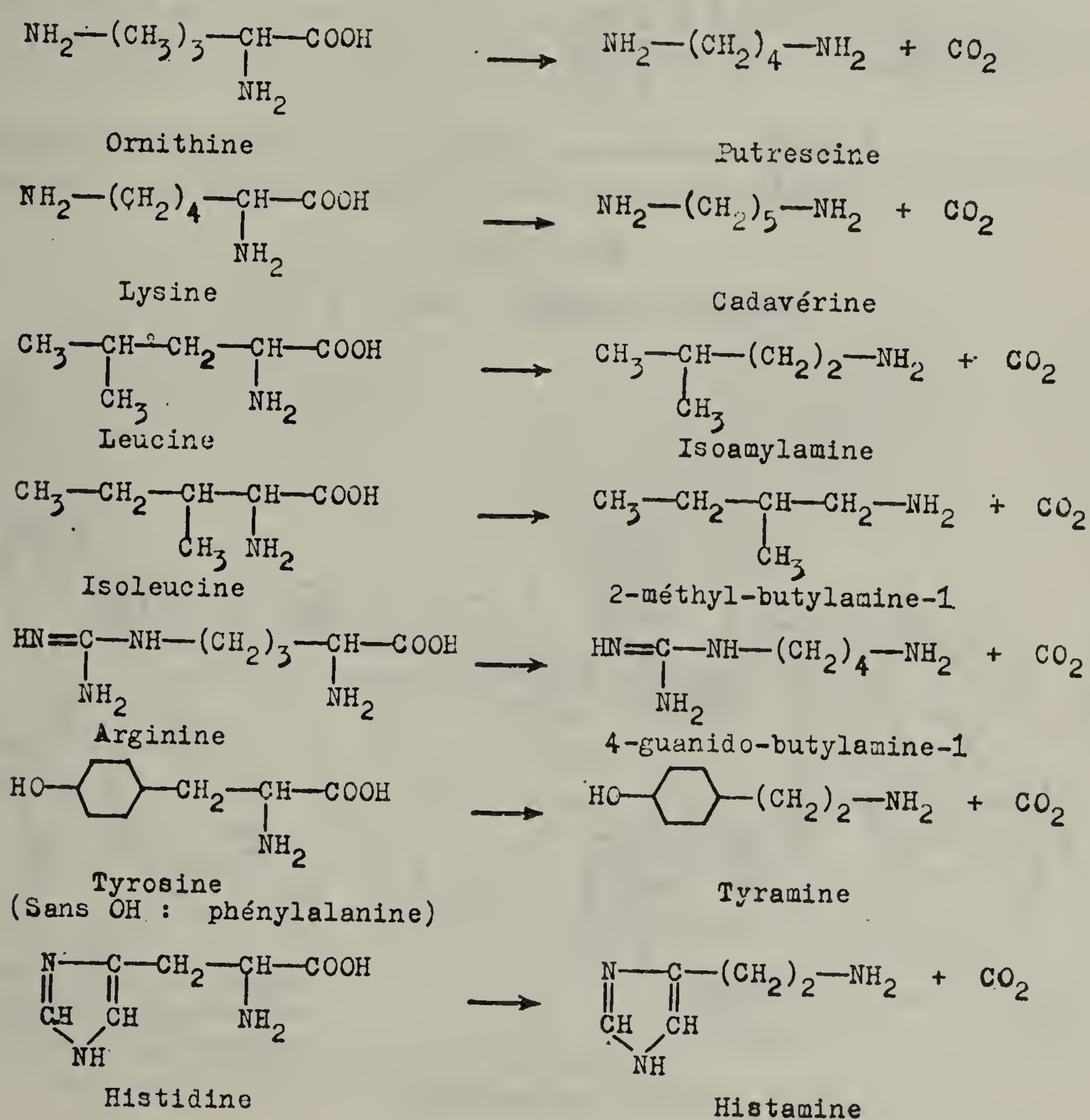

Fig. 30.

Acides α-aminés (à gauche) *et ptomaïnes* (à droite).

Dans les venins d'abeilles et de guêpes on trouve entre autres les poisons suivants:

Ornithine, lysine, leucine, isoleucine, arginine, tyrosine, phénylalanine, histidine.

Tous ces composés sont des acides α-aminés; ce sont des constituants des molécules protéiques, à partir desquelles ils naissent par hydrolyse.

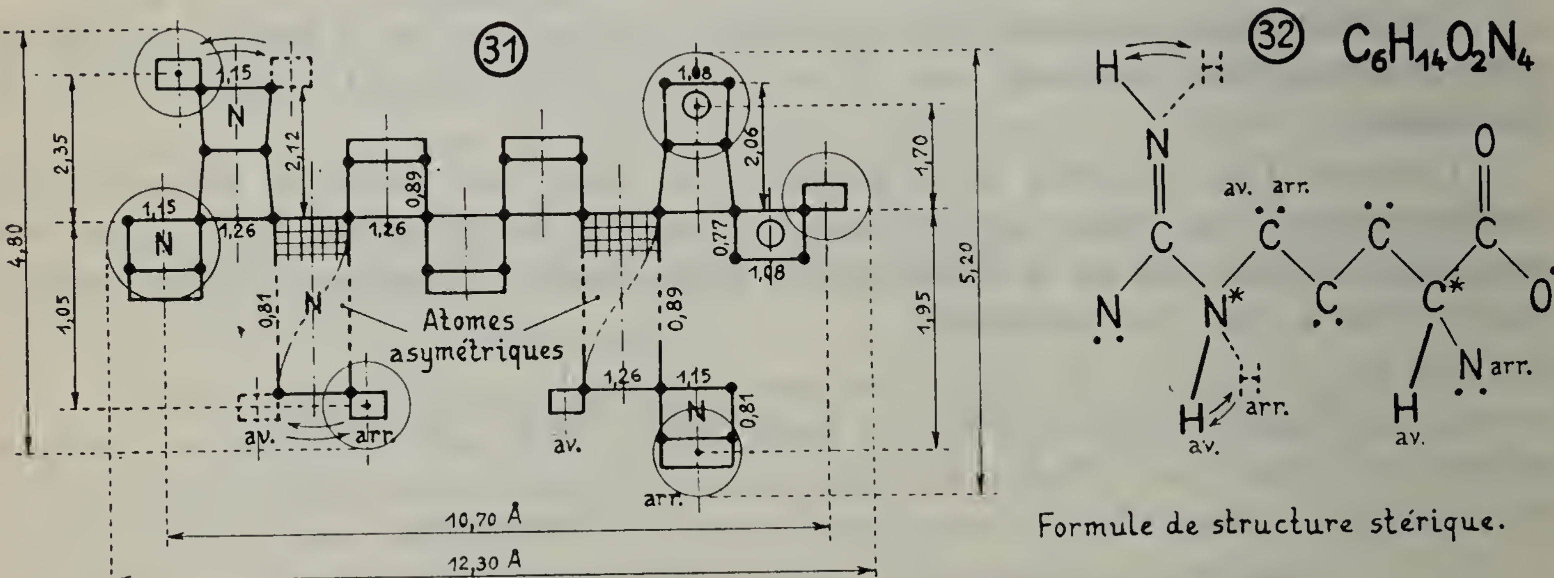

Fig. 31 - 32.

Arginine. - Echelle: 1 cm = 2 A.

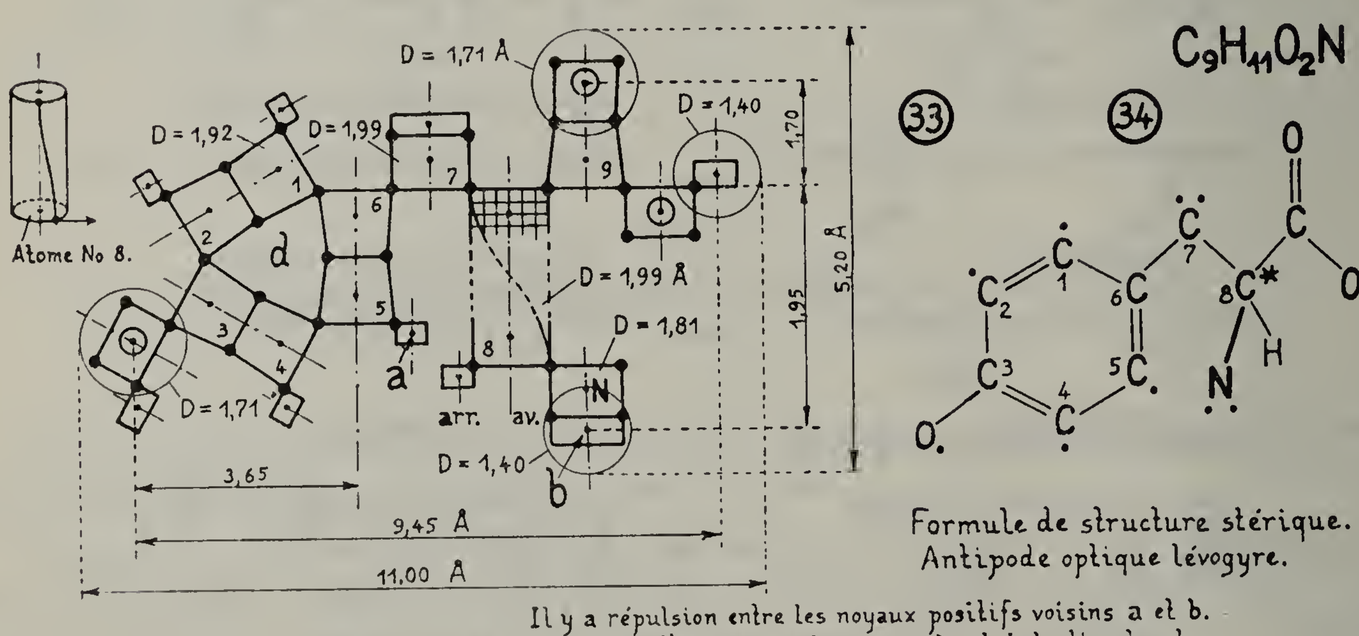

Fig. 33 - 34.

Tyrosine. Forme lévogyre. - En remplaçant OH par H, on a la phénylanaline.

Sous l'influence de bactéries de putréfaction, ces substances sont décarboxylées; les substances produites sont appelées ptomaïnes. Fig. 30.

L'action toxique des acides α-aminés et des ptomaïnes est liée au groupe CH—NH_2.

L'histamine (qui est une ptomaïne) se trouve dans le venin d'abeilles et de guêpes, et aussi dans celui de la salive de la punaise.

Nous nous limitons à indiquer les structures stéréoélectroniques des molécules des quatre composés suivants:

a) *Arginine*, fig. 31 et 32. La molécule possède deux atomes asymétriques, mais ceux-ci forment ensemble un seul centre d'activité optique, pour la raison indiquée à l'occasion de l'étude de la riboflavine (fig. 24). Les axes polaires de tous les atomes et les axes de spin de tous les électrons sont parallèles.

b) *Tyrosine*, fig. 33 et 34. La molécule possède un seul atome asymétrique.

c) *Histidine* et *histamine*, fig. 35 à 40. Pour l'histidine, même remarque que pour l'arginine. L'activité optique des molécules individuelles d'histamine n'est pas observable, les deux antipodes ne pouvant être séparés par suite du déplacement facile de l'atome d'H d'une arête à l'autre de l'atome d'azote. La cause de la non-observation d'une activité optique était restée inconnue jusqu'à ce jour.

Aux fig. 35, 36 et 37, nous avons représenté tous les atomes par leurs niveaux nodiques sphériques intérieurs et périphériques.

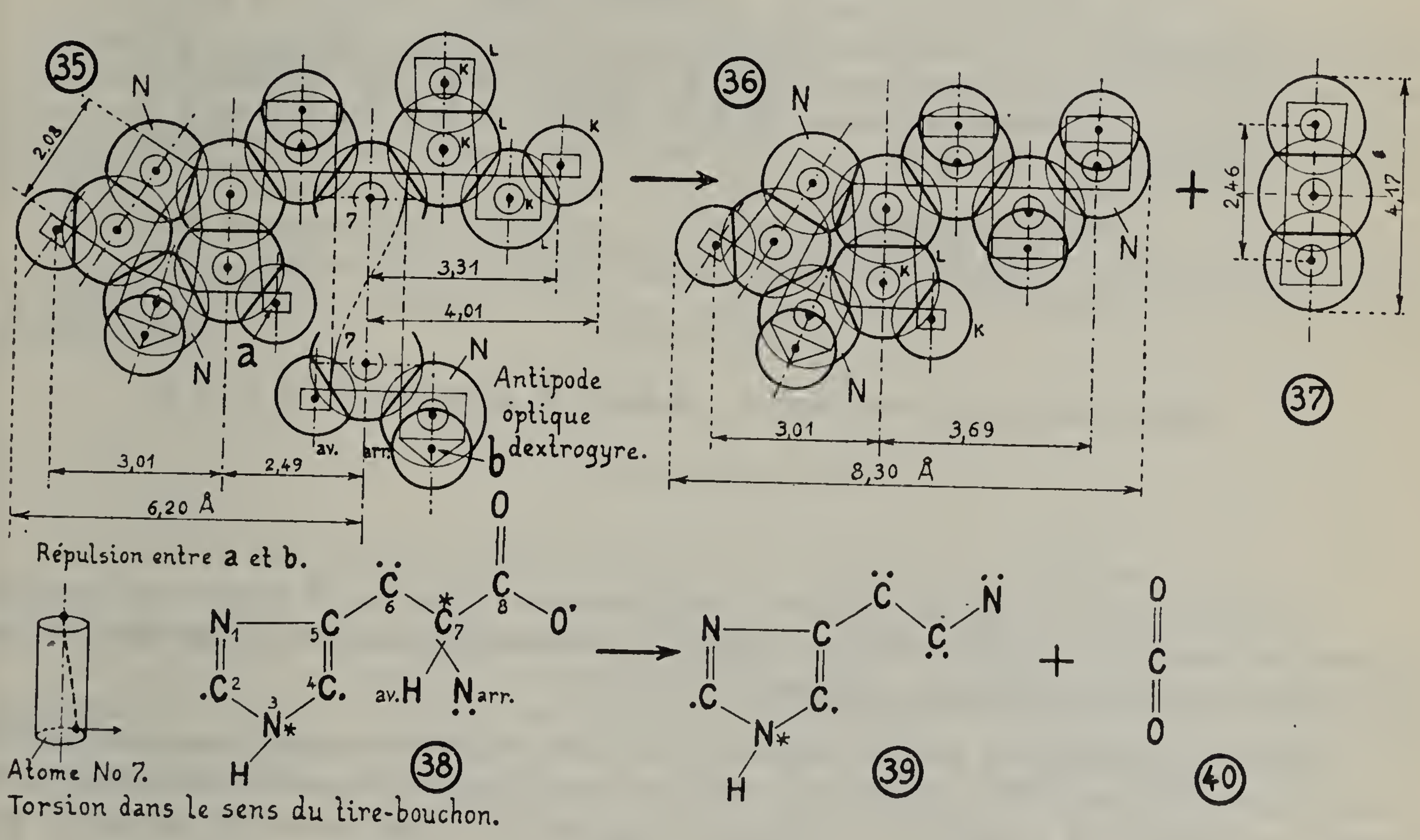

Fig. 35 - 40.

Histidine (forme dextrogyre) = *histamine* (optiquement inactive) + *acide carbonique.*

Un composé d'un très grand intérêt biologique est la *choline*, substance fort répandue dans le règne animal. Fig. 42. Au cours de la putréfaction elle est déshydratée en *neurine*, produit d'une grande toxicité; fig. 43. La choline exerce une action vaso-dilatatoire.

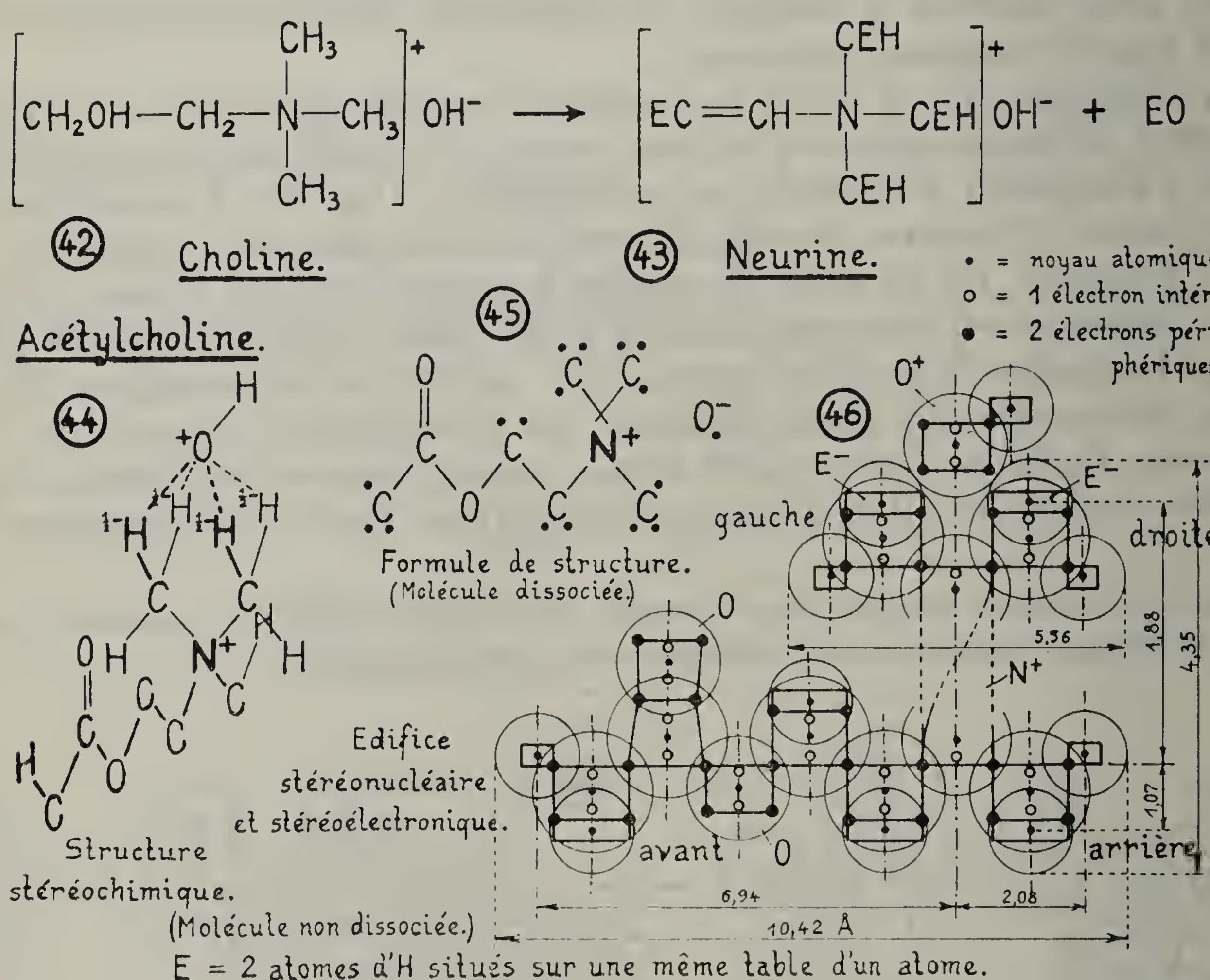

Fig. 42 - 46.

Choline, neurine et acétylcholine. - Echelle de la fig. 46: 1 cm = 2 A.

Le dérivé acétylé de la choline, l'*acétylcholine*, fig. 45, se trouve en grandes quantités dans le venin des frelons. Elle est beaucoup plus active que la choline. Structure stéréoélectronique intégrale, fig. 46.

Dans les molécules non dissociées des trois substances que nous venons de considérer, l'atome d'O du groupe OH est rattaché par quatre électrons de sa table normalement « franche » à quatre atomes d'H (fig. 44 et 46), de sorte que ces derniers sont des demi-ions négatifs, et que l'atome d'O est un ion positif. (Comparer: NH_4Cl). Lorsque le groupe OH se détache du reste de la molécule, l'atome d'O devient ion négatif, et les atomes d'H deviennent neutres.

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RÉSUMÉ

Un résumé de la théorie de l'atome à champ nodique est donné.

Dans les enveloppes atomiques de tous les éléments existe un champ électro-magétique à ondes stationnaires d'un seul et même mode universel de structure. Les électrons sont fixés à des noeuds électriques. Ce champ aussi bien que l'atome possède un axe de symétrie privilégié, de sorte que chaque atome possède à sa périphérie deux régions chimiques distinctes.

La laison chimique consiste en deux électrons périphériques communs aux deux atomes.

Comme application de la théorie, l'auteur décrit les structures stéréoélectroniques des molécules des poisons suivants: arginine, tyrosine, histidine, histamine, choline, neurine, acétylcholine.

RIASSUNTO

II. - Stereoelettronica dei veleni di Ape, Vespe e Calabroni.

Si riporta un riassunto della teoria dell'atomo a campo nodico.

Nel contorno atomico di tutti gli elementi esiste un campo elettromagnetico a onde stazionarie di un solo ed uguale modo universale di struttura. Gli elettroni sono fissati a nodi elettrici. Come l'atomo, anche questo campo possiede un asse di simmetria privilegiata, in modo che ogni atomo possiede alla sua periferia due regioni chimiche distinte.

Il legame chimico consiste in due elettroni periferici comuni ai due atomi.

Come applicazione della teoria, l'autore descrive le strutture stereoelettroniche delle seguenti molecole dei veleni: arginina, tirosina, istidina, istamina, colina, neurina, acetilcolina.

WECKERING R. (*)

III. - STEREOELECTRONIE DE POISONS DE FOURMIS ET DE COLEOPTERES

1. ACIDE FORMIQUE

Les sécrétions des fourmis de nombreuses espèces contiennent de l'acide formique. Chez *Formica rufa* L., la concentration en acide formique est de 21 à 71 %. On trouve cet acide aussi dans des chenilles. C'est le plus fort des acides gras; son groupe OH est très actif, de sorte qu'il est corrosif et vésicant. En tant qu'aldéhyde il est antiseptique et très réducteur.

Edifice stéréoélectronique intégral en perspective, fig. 52; structure stéréonucléaire avec indication de toutes les distances entre noyaux, fig. 53.

Sur la fig. 52 nous avons indiqué les orientations des axes privilégiés des noyaux et les orientations et directions des vecteurs de spin de tous les électrons.

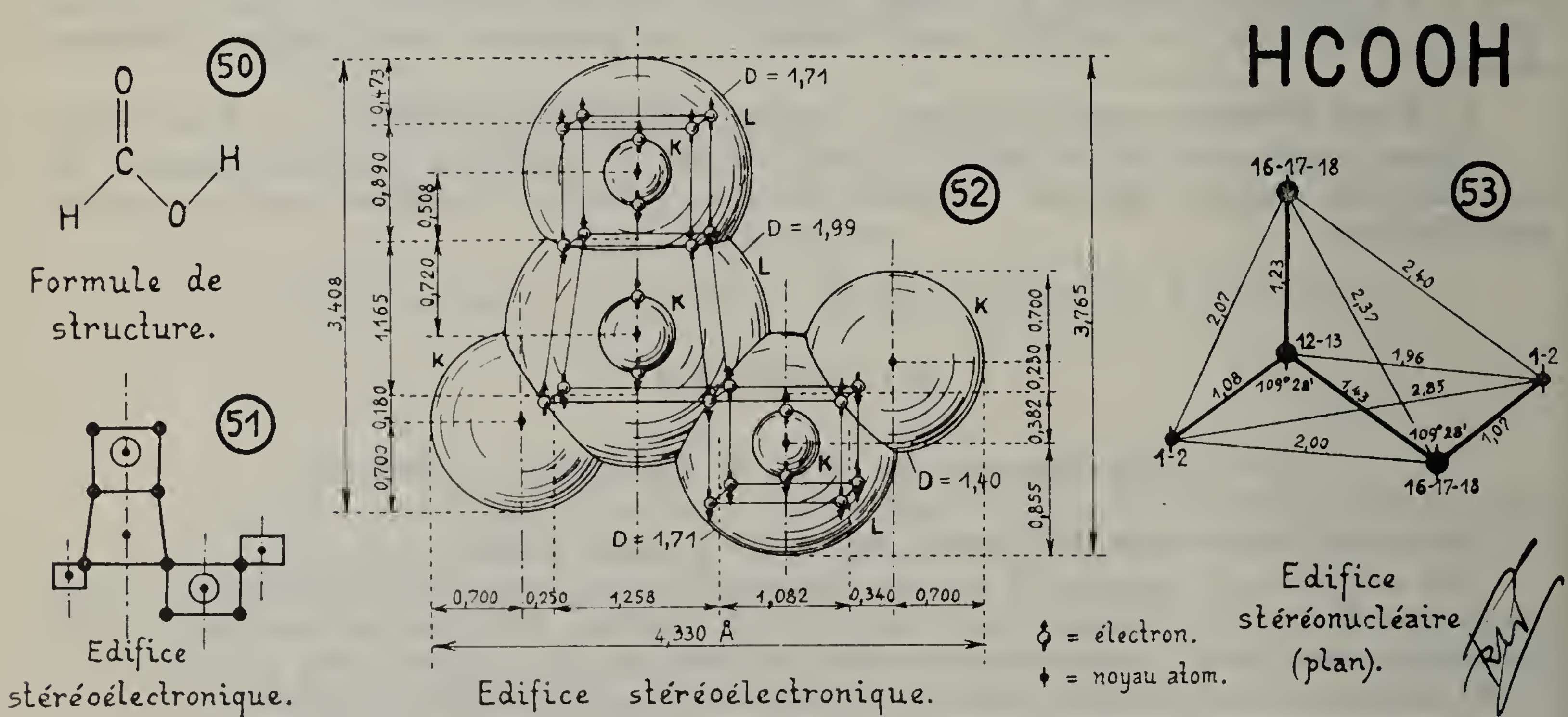

Fig. 50 - 53.
Acide formique. - Echelle: 1 cm = 0,9 A.

(*) *Ingénieur. Beho (Gouvy), Belgique.*

2. IRIDOMYRMÉCINE ET ISO-IRIDOMYRMÉCINES

Les petites fourmis tropicales *Iridomyrmex humilis* sécrètent par leur glande anale un venin, mortel pour beaucoup d'insectes, mais inoffensif pour l'homme. M. Pavan, de l'Université de Pavie, purifiait et cristallisait le venin en 1948, et le baptisa « iridomyrmécine ». La formule de structure, fig. 54, fut établie par Fusco, Trave et Vercellone.

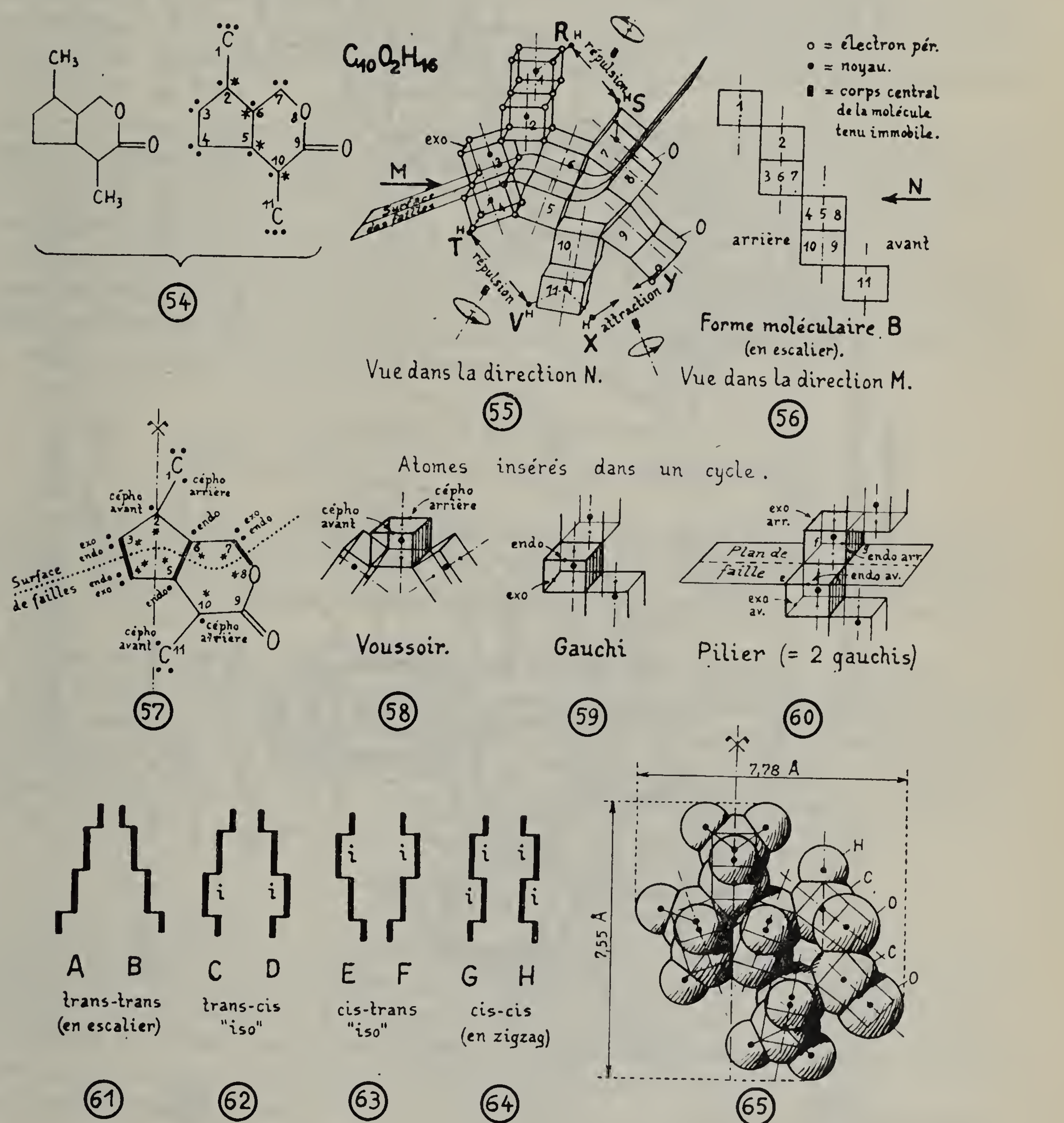

Fig. 54-65.

Iridomyrmécine.

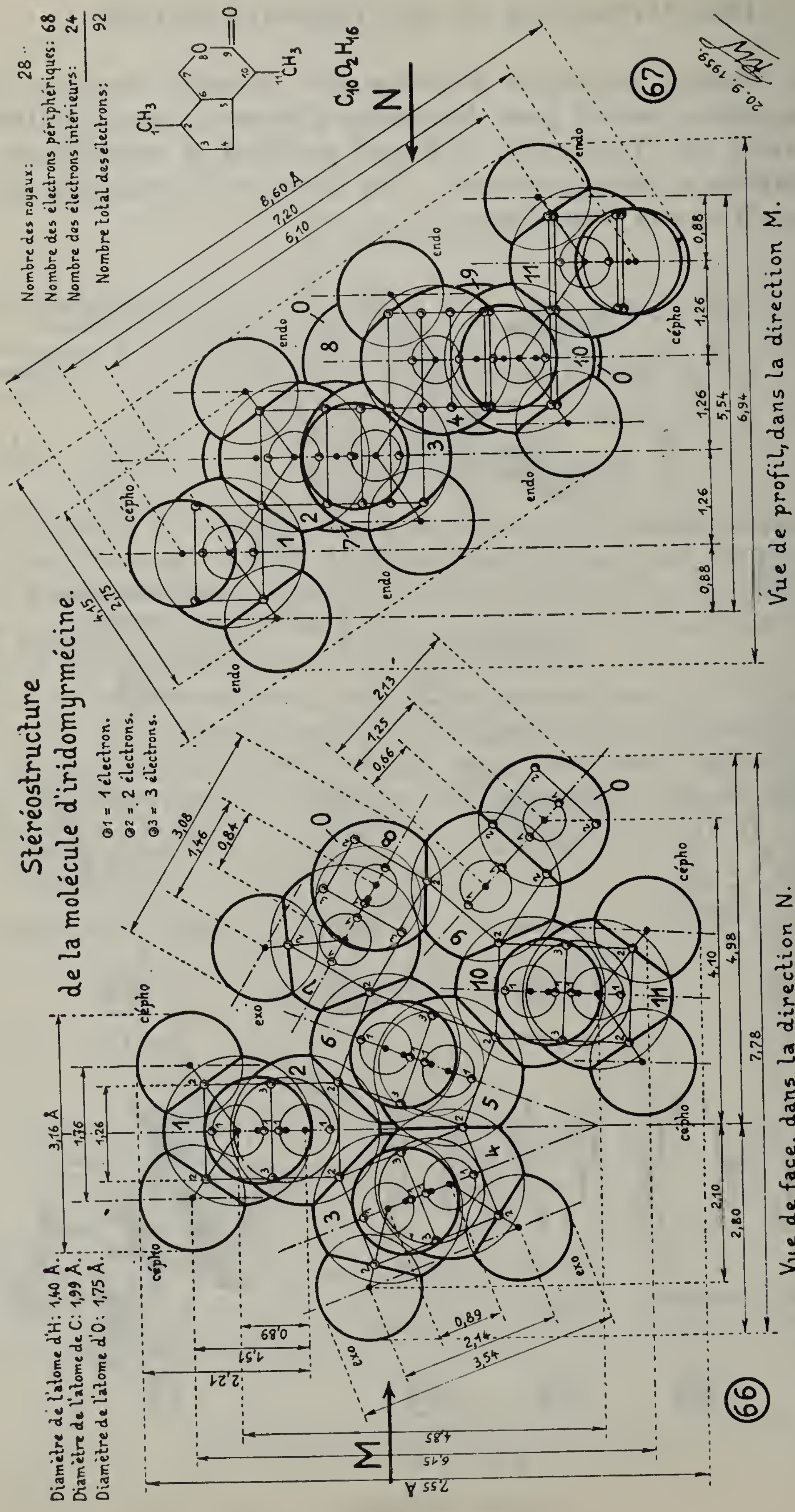

Fig. 66-67.

Iridomyrmécine. - Echelle: 1 cm. = 1,2 A.

La fig. 55 représente la stéréostructure du squelette bicyclique, les fig. 66 et 67, la structure stéréonucléaire et stéréoélectronique de la molécule, et la fig. 57, la formule de structure très detaillée qui s'en dégage.

Des considérations sur les forces d'attraction et de répulsion agissant entre les divers atomes et radicaux à la périphérie de la molécule (fig. 55), nous ont permis de conclure que la forme moléculaire B, fig. 55 et 65, correspond à l'antipode *lévogyre*; l'image dans un miroir correspond donc à la forme dextrogyre. A ce sujet relevons tout particulièrement que *jusqu'à présent il n'existait aucune méthode de détermination du sens de la rotation de la lumière polarisée dans une forme moléculaire asymétrique déterminée.* (Voir à ce sujet également les fig. 7, 22, 33 et 35).

Les six atomes contigus à la surface de faille, y compris les deux atomes 2 et 10, forment normalement ensemble un seul centre d'asymétrie (disposition en escalier qui est la plus stable, fig. 56 et 61). Si cependant l'un ou l'autre des deux radicaux CH_3 est retourné, on a deux composés « iso », fig. 62 et 63.

Les chercheurs australiens Cavill, Ford et Locksley ont extrait en 1956 une substance iso-iridomyrmécine des fourmis *Iridomyrmex nitidus* Mayr, et lui ont attribué la structure cis-trans, fig. 63. Il n'est cependant pas exclu, à notre avis, que le corps en question possède la forme trans-cis, fig. 62.

3. MÉTHYLHEPTÉNONE, PROPYL-ISOBUTYL-CÉTONE ET IRIDODIAL

Les deux premières substances prénommées fig. 68 et 70, furent extraites en 1955 par Pavan et Trave du venin des glandes des fourmis *Tapinoma nigerrimum* Nyl. Elles ont des propriétés insecticides. Structures stéréoélectroniques, fig. 69 et 71.

Ces deux savants ont extrait du même venin une troisième substance qui n'était pas encore connue jusque-là, et non vénéneuse: l'*iridodial*, fig. 72. Elle possède une certaine activité antibactérienne. On l'a encore trouvée dans *Iridomyrmex detectus* Sm. et dans *Iridomyrmex conifer* For. (Cavill, 1). Structure stéréoélectronique, fig. 73. Il y a deux centres d'activité optique: la faille et l'atome 6.

4. DENDROLASINE

Une huile incolore à odeur agréable fut isolée en 1956 par Pavan de la sécrétion des glandes mandibulaires de la fourmi *Dendrolasius fuliginosus* Latr.; il l'appellait « dendrolasine ». L'étude chimique en fut faite en 1957 par l'équipe Quilico, Piozzi et Pavan, qui concluait à la formule de structure fig. 74. Il s'agit d'un furane: β-(4,8-diméthyl-3,7-nonadiénil)-furane.

La dendrolasine est émise par l'animal irrité; il faut donc la considérer comme un venin protecteur.

La structure stéréoélectronique de la molécule, fig. 75, fait reconnaître que tous les atomes de C et d'O se trouvent dans un même plan.

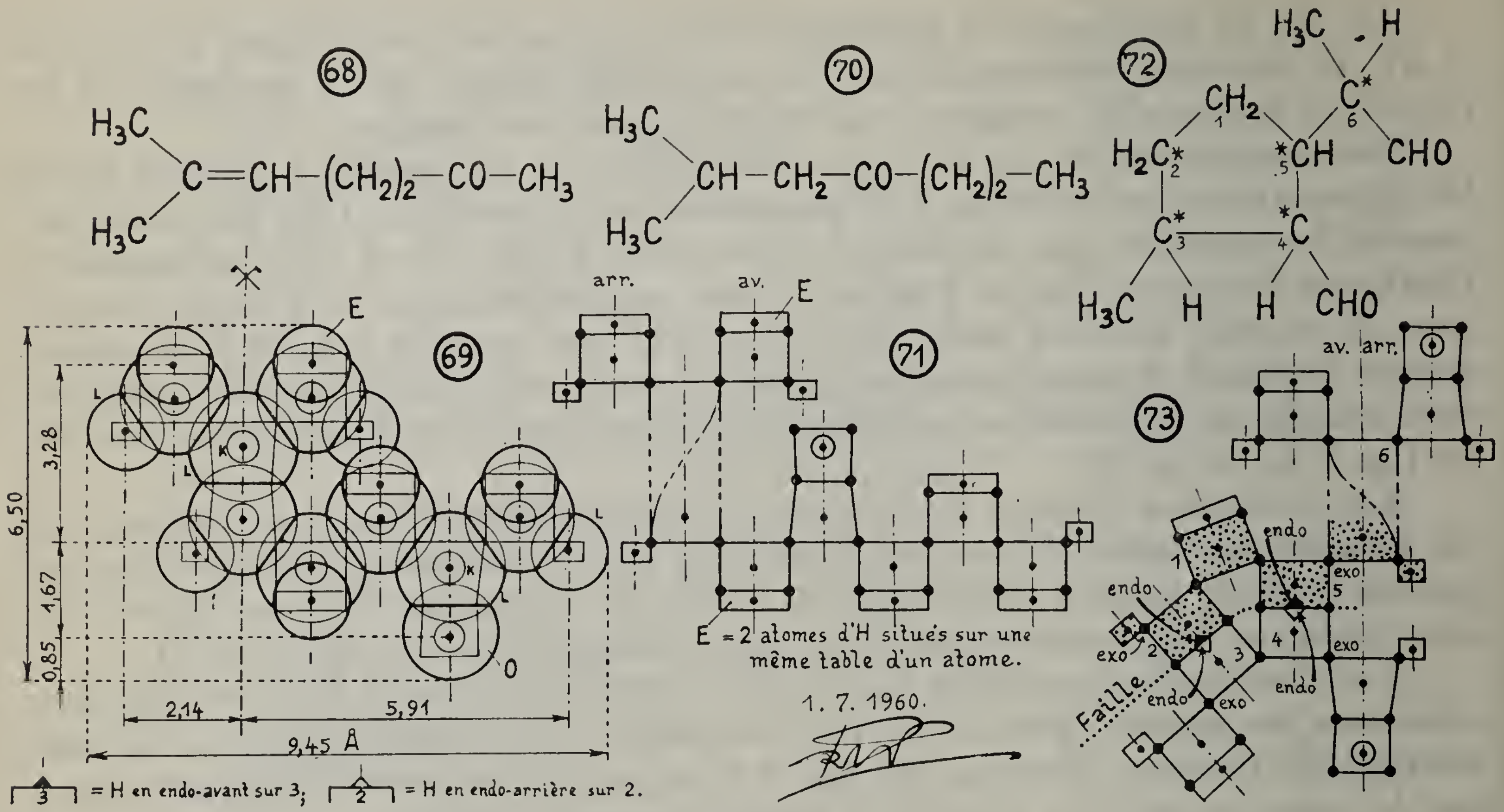

Fig. 68 - 73.
Méthylhepténone. - *Propyl-isobutyl-cétone.* - *Iridodial.* - Echelle: 1 cm = 2,1 A.

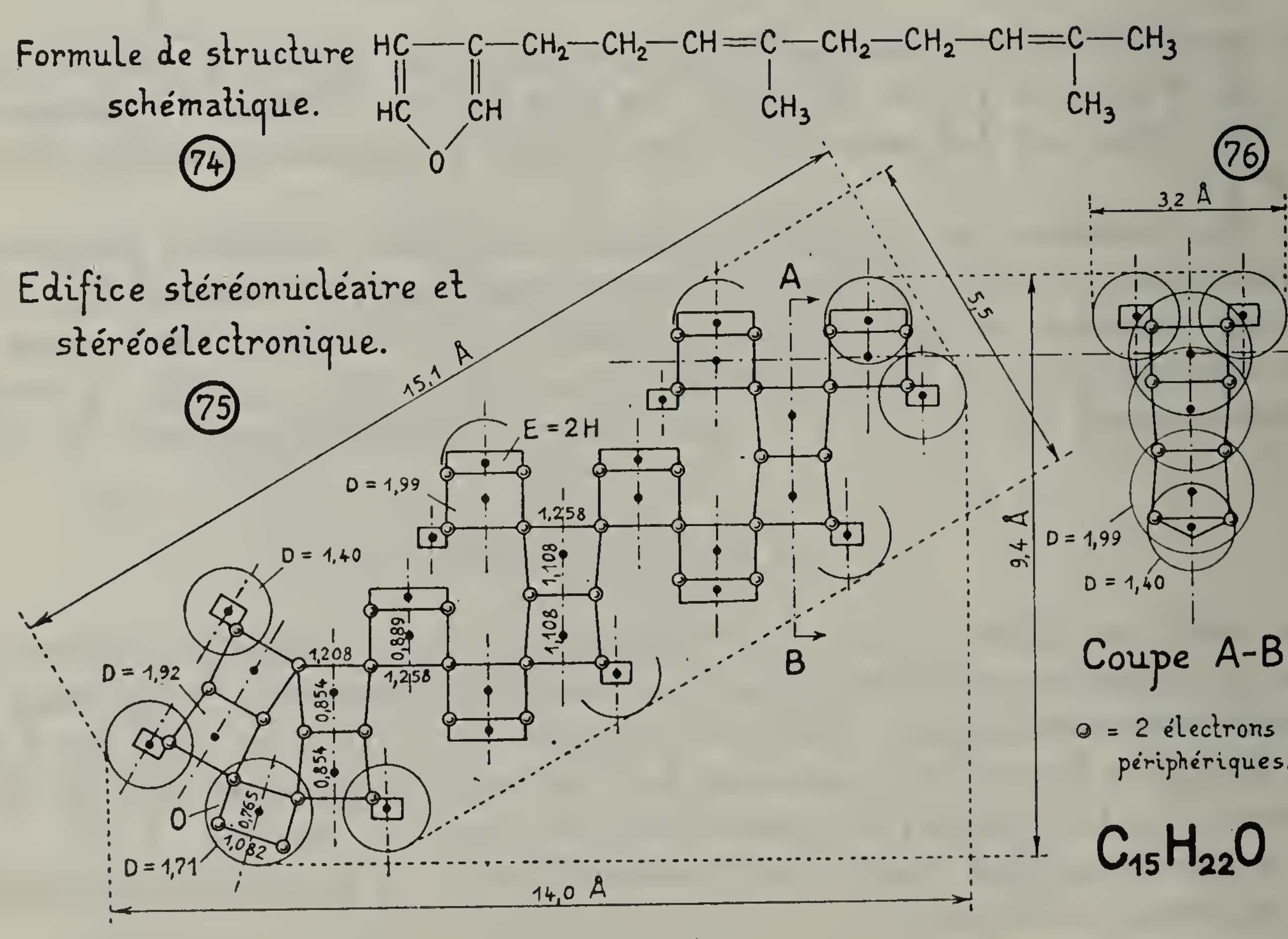

Fig. 74 - 76.
Dendrolasine. - Echelle: 1 cm = 2 A.

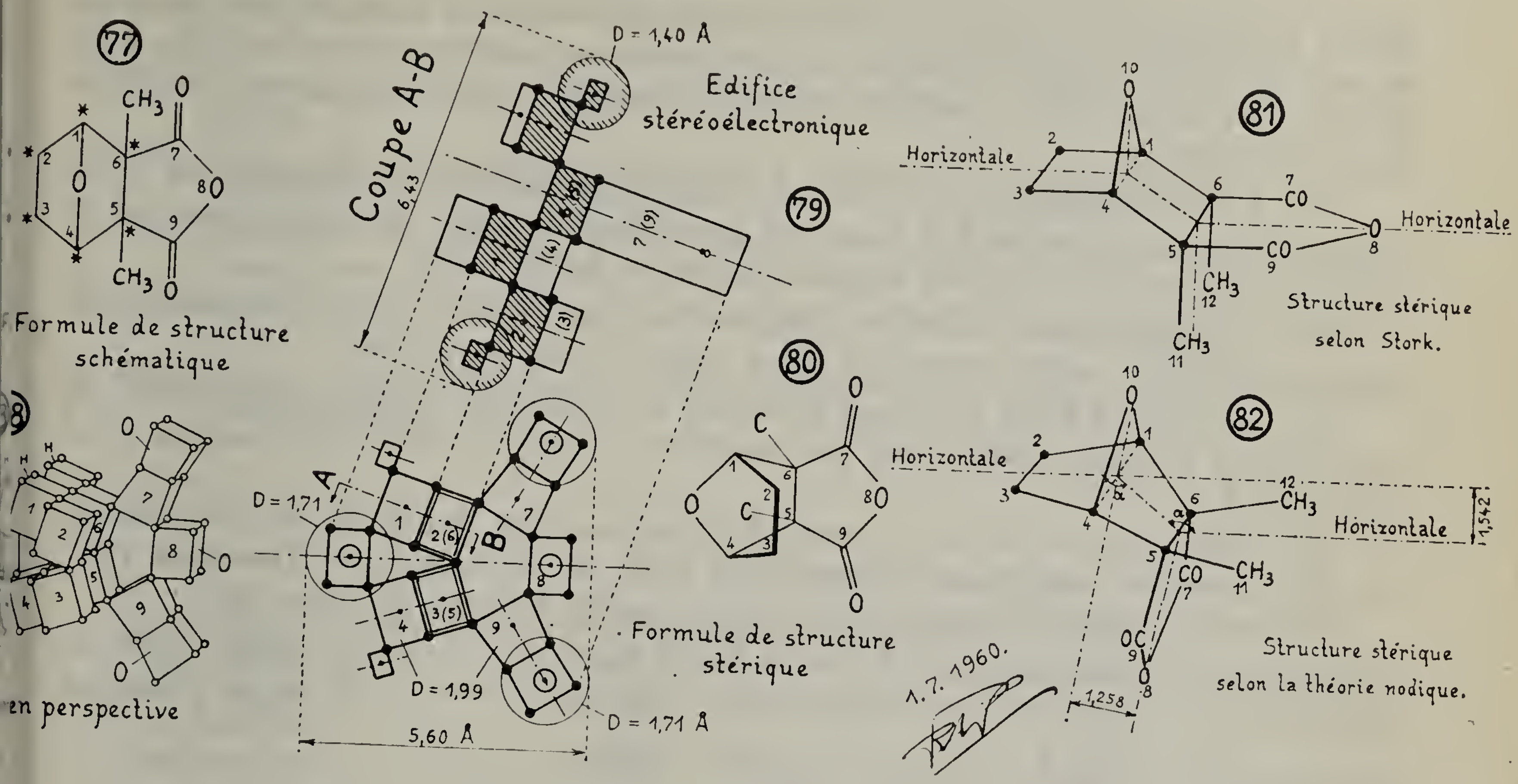

Fig. 77 - 82.
Cantharidine.

5. CANTHARIDINE

De divers coléoptères, notamment de ceux de l'espèce *Lytta vesicatoria* L., on retire la cantharidine, qui est contenue dans le sang de ces insectes. En cas de danger, ceux-ci éjectent le liquide sanguin corrosif et vésicant, ce qui les fait bénéficier d'un certain effet protecteur.

La cantharidine est un poison prononcé de cellules, pour beaucoup d'animaux aussi un poison de nerfs très actif.

Formule de structure, fig. 77. La molécule est optiquement inactive bien qu'elle contienne 6 atomes asymétriques, étant donné qu'elle possède un plan de symétrie. Le nombre des atomes asymétriques est reconnu sur l'édifice stéréoélectronique fig. 79.

Le cycle hexacarbonique est un cycle en bateau par nécessité stérique.

Avec la cantharidine nous tenons un exemple typique d'une substance apte à servir à la démonstration de *la surprenante capacité de la théorie atomique nodique d'élucider les conditions stériques dans les molécules.*

Les recherches effectuées par Stork et ses collaborateurs ont conduit à la structure stérique fig. 81. Or, celle-ci est incompatible avec les formes sté-

riques des atomes constitutifs et leurs modes d'association bien définis. En comparant cette figure avec la seule forme stérique possible, fig. 80 et 82, telle qu'elle résulte de notre édifice stéréoélectronique, nous constatons les différences suivantes:

1° - Les connexions 2-1 et 3-4 ne sont pas parallèles, la distance 1-4 étant plus grande que celle de 2-3. Il en est pareillement pour les connexions 1-6 et 4-5, pour celles de 6-7 et 5-9, et pour celles de 6-12 et 5-11.

2° - Le plan 1-10-4 n'est pas perpendiculaire à celui de 1-2-3-4, mais bissecteur de l'angle solide entre les deux plans 1-2-3-4 et 1-6-5-4.

3° - Le plan 1-2-3-4 n'est pas parallèle à celui de 6-7-8-9-5, mais à celui de 6-12.5-11, et le plan 1-10-4 n'est pas parallèle à celui de 6-12.5-11, mais à celui de 6-7-8-9-5.

La distance entre les deux plans horizontaux sur la fig. 82 est de 1,542 A, et celle entre les deux plans obliques, de 1,258 A. L'angle α est d'environ 109°.

Distances entre les centres des atomes, selon les fig. 79, 80 et 82:

1- 2 et 3- 4 : 1,54 A.	5-9 et 6-7 : 1,45 A.	1-4 et 11-12 : 2,00 A.
1- 6 et 4- 5 : 1,54 A.	7-8 et 9-8 : 1,38 A.	8-11 : 4,10 A.
6-12 et 5-11 : 1,54 A.	2-3 et 5-6 : 2,00 A.	

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RÉSUMÉ

Les structures stéréoélectroniques sont données des composés suivants:

Acide formique. Sur la figure sont indiquées les orientations des axes privilégiés des noyaux atomiques de la molécule et les orientations et directions des vecteurs de spin de tous les électrons.

Iridomyrmécine. La structure revenant à chaque antipode optique de ce composé est reconnue par le fait que celle de l'un d'eux est déformée par de la torsion à droite, celle de l'autre, par de la torsion à gauche.

Méthylhepténone, propyl-isobutyl-cétone et iridodial.

Dendrolasine. Molécule plane.

Cantharidine. L'auteur démontre à l'aide de ce composé la surprenante capacité de la théorie atomique nodique d'élucider les conditions stériques dans les molécules.

RIASSUNTO

III. - Stereoelettronica dei veleni di Formiche e di Coleotteri.

Sono riportate le strutture stereoelettroniche dei seguenti composti:

Acido formico. Sulle figure sono indicate le orientazioni degli assi privilegiati dei nuclei atomici della molecola e le orientazioni e direzioni dei vettori di spin di tutti gli elettroni.

Iridomirmecina. La struttura, che ritorna a ogni antipode ottico di questa sostanza, è riconosciuta per il fatto che quella di uno di essi è deformata da una torsione a destra e quella dell'altro da una torsione a sinistra.

Metileptenone, propilisobutilchetone, iridodial.

Dendrolasina. Molecola piana.

Cantaridina. L'autore dimostra con l'aiuto di questo composto la sorprendente capacità della teoria atomica nodica di chiarire le condizioni steriche nelle molecole.

Eisner T., Meinwald J., Monro A., Ghent R. (*)

THE DEFENSIVE SPRAY OF A WHIPSCORPION (1)

Whipscorpions of the pedipalp family *Thelyphonidae* have long been known for their ability to discharge a pungent liquid spray when disturbed. The chemical nature of the secretion has never been determined, nor has any definite evidence been presented in favor of the long-accepted view that the spray serves in defense against predators. The purpose of this paper is to summarize briefly the behavioral, physiological, and chemical work done recently in our laboratories and under field conditions on a large whipscorpion of the southwestern United States: *Mastigoproctus giganteus* (Lucas). The details of the study will be presented at length elsewhere. It will suffice here to mention only the main techniques and results.

THE SPRAY MECHANISM

The glands have been described by Börner (1904) and others. The two elongate sacs are positioned lengthwise ventrally in the opisthosoma and open close together next to the anus at the tip of the short postabdominal knob forming the base of the flagellum. Each sac is enveloped by muscles, the contraction of which effects the discharge.

A series of experiments were made to determine with some degree of precision characteristics of the spray such as its direction, range, and degree of dispersion. The technique was essentially the same as that used previously with bombardier beetles and other insects having defensive sprays (Eisner 1958 a, 1058 b, Eisner et al. 1959). Individual whipscorpions were affixed to rods, placed on sheets of filter paper stained purple with alkaline solution of phenolphtalein,

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and then caused to spray on this paper by subjecting them to mild traumatic stimuli locally applied. The acid spray decolors the indicator, producing a dense pattern of small white dots wherever the paper is hit.

The spray is discharged with considerable force, commonly reaching a distance of 20 to 40 cm. It is in a very fine state of dispersion, the individual droplets having been estimated to average below 0.005 μl. Of special interest was the finding that the spray can be ejected in almost any direction, and that it is invariably aimed with great precision toward the particular appendage or region of the body subjected to stimulation. In aiming, the animals adjust the opisthosoma and revolve the postabdominal knob in such a way as to direct the glandular opening toward the stimuls. Marksmanship if this sort is by no means restricted to whipscorpions. In fact, it seems to be the rule rather than the exception in arthropods with defensive sprays. We have been able to demonstrate it in most of the animals we worked on, including bombardier beetles (Eisner 1958 a), some cockroaches (Eisner 1958 b, Eisner et al. 1959), and a variety of earwigs, walking sticks, notodontid caterpillars, pentatomid and coreid hemiptera, and others (Eisner unpublished). These insects do not necessarily all aim as precisely as do whipscorpions, but they nevertheless are able to control to some extent the direction in which a discharge is produced.

EFFECT OF THE SECRETION ON PREDATORS

Predator-prey encounters were observed, involving whipscorpions and a variety of invertebrate and vertebrate predators, including ants [*Pogonomyrmex occidentalis* (Cresson)], solpugids (*Eremobates* sp.), lizards (*Anolis carolinensis* Voigt, *A. sagrei* Cocteau), birds [*Cyanocitta cristata* (L.), *C. stellari* (Gmelin)], grasshopper mice (*Onychomys torridus* Coues), and an armadillo (*Dasypus novemcinctus* L.). Most of the encounters took place in the laboratory, but the experiments with *Pogonomyrmex* were made just outside the nest entrance of a natural colony.

The spray proved to be a defensive device of extraordinary effectiveness. As long as a whipscorpion had not exhauted its secretory supply, it was virtually invulnerable. All predators were repelled. The tests also showed clearly the extent to which the ability to aim improves the effectiveness of the spray. Small predators such as the ants, which might otherwise have been missed, invariably bore the full impact of an aimed discharge. Aiming proved of advantage even against some larger predators such as the birds. Since the birds initiated their attack by pinching and seizing the whipscorpion with the bill, the discharge was always directed so as to douse much of the head; some spray reached the eyes, and this had an immediate effect.

It should be mentioned that not all the predators tested are likely natural enemies of whipscorpions, but this limitation should not detract from the basic finding that the spray is one of very general protective effectiveness.

THE CHEMICAL NATURE OF THE SECRETION

Samples of secretion were obtained in pure form simply by causing the animals to spray directly into small glass vials. Sufficient amounts were gathered to permit a very precise determination of the nature and proportions of its components. Standard physical and chemical techniques were used, based on infrared spectroscopy, gas chromatography, and preparation of crystalline derivatives. The analysis found:

84 %	Acetic Acid
5 %	Caprylic Acid
11 %	Water

THE SPECIAL SIGNIFICANCE OF CAPRYLIC ACID

Although the principal active constituent of the spray is obviously the acetic acid, a series of experiments were carried out that demonstrated that the caprylic acid is not merely of incidental significance, but has an important ancillary function in improving the effectiveness of the secretion against arthropod predators. Specifically, the caprylic acid was shown to have a dual role: 1) it acts as a wetting agent, promoting the rapid spread of the spray droplets over the cuticle of the predator, and thereby increasing the effective area of contact of the poison, and 2) it serves to exert a marked accelerating effect on the penetration of the mixture through the cuticle.

The spreading effect was demonstrated simply by placing droplets of acetic acid, with and without coprylic acid, on isolated pieces of cockroach cuticle. The droplets without caprylic acid remained globular in shape until evaporated, while those with caprylic spread rapidly and completely over a wide area.

The penetration-accelerating effect was demonstrated first by way of a simple permeability study involving isolated pieces of cockroach cuticle. Droplets of acetic acid, with and without caprylic acid, were placed on the cuticle, and their passage through the cuticle into an underlying gel impregnated with indicator was timed with a stopwatch. The droplets containing the caprylic acid invariably penetrated faster than did those without it.

A second series of experiments, also designed to test the penetration-promoting effect, and serving primarily as a check to the permeability test, was done on live animals. One of the experiments consisted in placing droplets of acetic acid, again either with or without caprylic acid, onto the abdomen of cockroaches, and timing the onset of the stereotyped scratchreflex induced. As was to be expected, onset was earliest with the mixtures containing caprylic acid. The other experiment also gave results according to prediction. Fly larvae were immersed in the acid mixtures, and the duration of their activity until complete immobilisation was timed. They wiggled longest in the mixture containing acetic acid alone.

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SUMMARY

The whipscorpion *Mastigoproctus giganteus* (Lucas) has a pair of voluminous glands the secretion of which consists of 84 % acetic acid, 5 % caprylic acid, and 11 % water.

The secretion is ejected forcibly as a finely dispersed spray that can be aimed accurately in virtually all directions. It acts as a strong deterrent to predator attack, being repellent to both arthropods and vertebrates.

The presence of caprylic acid considerably improves the effectiveness of the weapon as it is used against arthropods. By acting as a wetting agent it promotes the spread of the secretion over the cuticle of the predator, and at the same time it facilitates the penetration of the mixture through the cuticular barrier.

RIASSUNTO

Lo spruzzo difensivo dell'Aracnide Pedipalpe Mastigoproctus giganteus (*Lucas*).

L'Aracnide Pedipalpe *Mastigoproctus giganteus* (Lucas) possiede un paio di glandole voluminose la cui secrezione consiste dell' 84 % di acido acetico, 5 % di acido caprilico, 11 % di acqua. La secrezione è emessa impetuosamente sotto forma di spruzzo ben disperso che può essere diretto con precisione praticamente in tutte le direzioni. Ha una forte azione nell'impedire l'attacco dei predatori avendo azione repellente sia sugli Artropodi che sui Vertebrati. La presenza di acido caprilico aumenta considerevolmente l'efficacia dell'arma, particolarmente quando è usato contro gli Artropodi. Agendo come arma da spruzzo promuove lo spandersi della secrezione sopra la cuticola del predatore e allo stesso tempo facilita la penetrazione del veleno attraverso la barriera cuticolare.

FIG. 1. *Mastigoproctus giganteus* in ventral view with opisthosoma dissected open to show the two glandular sacs.

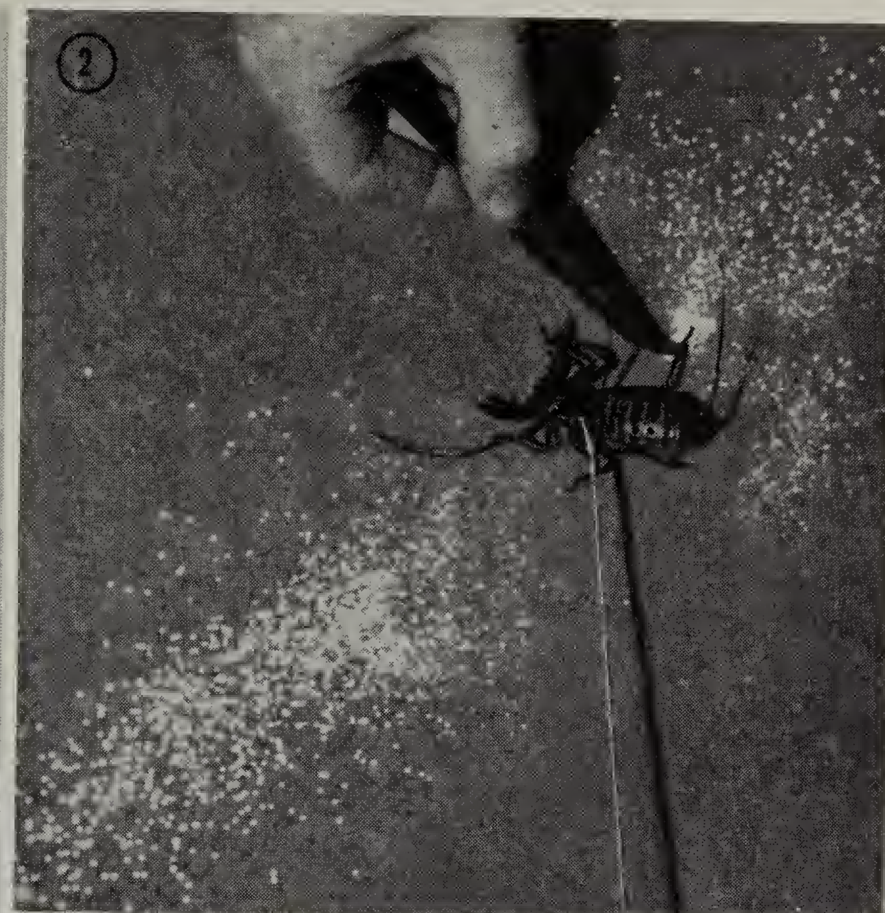
FIG. 2. Whipscorpion that has been caused to spray twice on alkaline phenolphthalein paper. The first discharge (lower left) was elicited by pinching the left third appendage. The second discharge is being produced in response to pinching the last leg on the right.

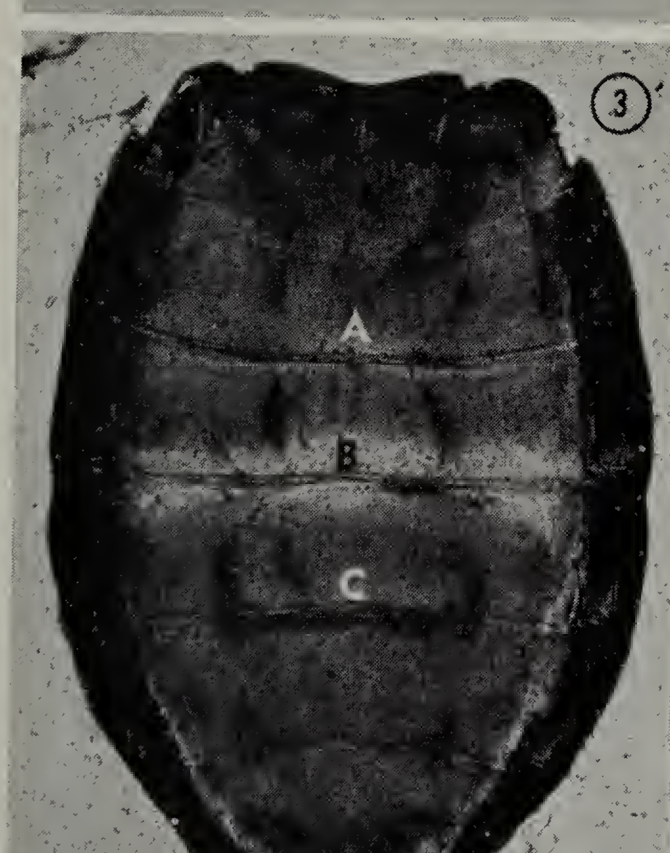
FIG. 3. Surface view of an isolated piece of cuticle (dorsal abdominal shield of *Rhodnius prolixus*) mounted on a layer of gelatin stained dark red with alkaline phenolphthalein. A mixture of acetic and caprylic acid has been applied to the intersegmental membrane at B; the mixture has passed through the membrane and has destained the indicator. By contrast, the solutions containing acetic acid alone which were applied to the membranes at A and C failed to penetrate the cuticle.

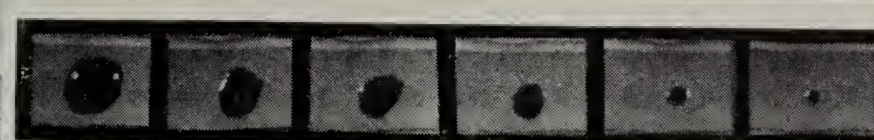
FIG. 4. Successive stages in the evaporation of a droplet of acetic acid placed on abdominal cuticle of a cockroach.

FIG. 5. Same as figure 4, but the droplet in this case contains 5 % caprylic acid. The caprylic acid promotes the spread of the droplet. (For purposes of photography the droplets in figures 4 and 5 have been stained with a trace of crystal violet).

FIG. 6. A grasshopper mouse scurrying away and digging its muzzle into the sand after being sprayed during an attack on the whipscorpion shown on the left.







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Bettini S., Toschi-Frontali N. (*)

BIOCHEMICAL AND TOXICOLOGICAL ASPECTS OF *LATRODECTUS TREDECIMGUTTATUS* VENOM

The importance of *L. tredecimguttatus* as a venomuos spider in Italy can be gathered by the fact that during the years between 1938 and 1958 the number of cases of latrodectismus in 4 provinces of central Italy amounted to about one thousand (2, 3, 6). An immune serum is being produced by our Institute (5, 7, 36). In the present note some preliminary work undertaken by our Laboratory on the biochemistry and toxicology of this spider's venom is reported.

The venom *in toto* was prepared by dissecting out the venomous glands which were homogenized in saline and centrifuged at 3000 rpm for 15 minutes.

The LD_{50} values on guinea pig and mouse were obtained following Reed and Muench's method (37), through subcutaneous injections and by counting mortality at 24 hrs (guinea pigs weighed 350-400 g, mice 13-17 g). The same method was followed on *Periplaneta americana*: male roaches were injected into the abdomen according to a technique already described (4); mortality counts were obtained at 48 hrs (roaches weighed 0.70-0.80 g). Because *P. americana* individuals can be obtained only in limited numbers, due to the relatively long life cycle of the species, we thought it more convenient to use for toxicity tests an insect that could be obtained in large numbers throughout the year. Considering the fact that Wiener and Drummond (44) used with success *Drosophila* in testing the toxicity of *L. hasseltii* venom, we worked out a test on *Musca domestica* which proved to be a rather useful one. We have employed for this purpose the strain « Switzerland » (susceptible to the common insecticides) bred in our Laboratory following the routine technique. Female flies have been injected with volumes equal to 0.25-0.50 μl into the thorax by means of a microsyringe. Flies weighed 22-25 mg each. The LD_{50} values are reported on Table I, where it can be observed that mouse and *Musca* (female) show LD_{50} values of the same order, while *P. americana* (male) shows to be about 20 times more tolerant, and guinea pig 12 times more susceptible than mouse. Wiener and Drummond (44) found that *L. hasseltii*'s venom is approximately 8 times

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more toxic on *Drosophila* than on mouse. The toxicity of *L. tredecimguttatus* venom on mouse is of the same order as that of *Phoneutria fera* (*Ctenus ferus* and *C. nigriventer*) and *Scaptocosa (Lycosa) raptoria*, while that of all other spiders so far experimented on is much lower, as shown by Bücherl (8, 9, 10).

The venom diluted in saline, at 4° C, lost its toxicity slowly with time: in about 35 days the venom's toxicity decreased to 1/4 of its original value. At — 15° and at — 25° C the toxicity remained constant for at least 35 days. At room temperature, however, the venom lost completely its activity on the third day. Tests were carried out on house flies.

It has been shown by Lebez (30, 31) that *L. tredecimguttatus* venom, collected through cotton plugs, has a high proteolytic (as well as glycogenolytic) activity. Cantore and Bettini (13) observed that the venom of dissected glands, either fresh or dry, showed no proteolytic activity (on gelatine), while the venom collected through cotton plugs, or the direct spider bite on gelatine film, showed a marked proteolytic activity. These apparent contradictory results between the venom collected from the mouth opening and that from the glands directly, could be explained by the fact that in the first case the venom might have been contaminated by enzymes secreted by the digestive apparatus. Kaiser (26, 27, 28) has reported proteolytic activity by *S. raptoria* and *P. nigriventer* pure venoms on casein and fibrin. (It would be interesting to test *Latrodectus* venom on these substrates as well).

As previously reported by *Lebez* (30, 31) and by Cantore and Bettini (1) the venom shows no hemolytic activity. Analogous results were also noted by Troise (43) on *L. mactans*, by Finalayson (19) on *L. indistinctus* and by Kaiser (27) on *S. raptoria* and *P. nigriventer*.

The venom *in toto* is very active on rabbit's isolated ileum. A concentration of 0.5 mg of dry glands/l produces a strong contraction (12). This effect is shown to a less extent also on *Cybister lateralimarginalis* isolated foregut (35) which increases in tonus at a concentration of 362 mg of dry glands/l. No effect was detected on guines pig's isolated uterus by Troise (43) and Sampayo (39) (*L. mactans*), by Shapiro et al. (41) (*L. indistinctus*) and by Cantore (12) (*L. tredecimguttatus*). Shapiro et al. (41) found that the venom enhanced the guinea pig uterine contractions during delivery; Cantore (12) found no effect on the gravid uterus.

The venom of 3 species of *Latrodectus* (*L. tredecimguttatus, L. mactans* and *L. indistinctus*), as already demonstrated on mammals by Troise (43), Shapiro et al. (41), Sampayo (39, 40), Suarez et al. (42), Cicardo (17), Calvo et al. (11), and Cantore and Bettini (15), produces a mild arterial hypertension which reaches its maximum after approximately 6 minutes.

The bronchospastic activity of the venom, already shown *in vivo* by Houssay and Negrete (21), Kellaway (29), Sampayo (39, 40), and Cantore and Bettini (14), has been studied *in vitro* in the present research, employing the method of Castillo and De Beer (16) by which a series of guinea pig tracheal rings are tied together to form a chain which is suspended in a saline bath at 35° C.

Through this technique it was possible to obtain a positive effect at a venom concentration of 32 mg of dry glands/l. This method, however, is much less sensitive than the *in vivo* one where a dose of 0.020 mg/Kg produces a marked increase of the bronchial muscles tonus (14).

Lebez (30, 31) has reported that the venom collected through cotton plugs inhibits the erythrocyte cholinesterase, while Cantore and Bettini (13) testing on dissected glands have reported a neglectable degree of inhibition on *Torpedo* electric organ cholinesterase. We have repeated the experiment on the same material and have obtained negative results only. These contradictory results could be justified, as already stated, by considering the differences in the venom collecting procedure, for the material collected through cotton plugs might have contained small amounts of digestive enzymes responsible perhaps for the inactivation of cholinesterase.

A hyaluronidase-like activity of the venom *in toto* has already been shown *in vivo* and *in vitro* (conc. 0.070 g/l) by Cantore and Bettini (13). The venoms of *P. nigriventer* and *S. raptoria*, as reported by Kaiser (24, 25, 26, 27), show the same property. According to this author, the hyaluronidase splitting enzyme (arachnomucinase) of these species is inhibited by heparine, by sulfonated pectins (25) and by human serum (24), behaving in this respect similarly to testicular hyaluronidase. No hyaluronidase-like activity was reported by Jaques (22, 23) from *Araneus diadematus* venom.

In order to acquire some knoledge on the various components of the venom and on their relation to toxicity and pharmacological activities, the following experiments were undertaken.

An analysis of the free amino acids of the venom, employing Rockland and Underwood's paper chromatographic method (38), showed the following amino acids to be present in higher amounts: taurine, glutamic acid, glutamine, alanine, arginine followed by glycine, γ-aminobutyric acid, aspartic acid, asparagine, leucine, histidine, lysine. According to Fischer and Bohn (20), glutamic acid and γ-aminobutyric acid are the most abundant amino acids present in venoms of 9 other spider species.

The venom, dialized against saline solution, as already reported by D'Amour et al. (18) on rat and by Cantore and Bettini (13) on guinea pig, showed no decrease in toxicity. We have repeated the test on *Periplaneta* and on *Musca*, and the same results were obtained.

Lebez (30) reported « relatively great quantities of lipoids (probably lipoproteins) » to be present in the venom; however, Cantore and Bettini (13) were unable to detect any lipo-proteins employing the paper electrophoresis method with Sudan-Black B staining. Further work is to be devoted to this problem.

Six protein fractions of *L. tredecimguttatus* venom were first separated by Muic *et al.* (34) who had collected the venom using cotton plugs. Cantore and Bettini (13) found 5 components by employing an extract of dissected glands. In the present research we have employed the latter's method (borate solution of Adjuvantis, pH 8.6 and ionic strength 0.07; 14 hrs run at 15° C). An extract

of 50 couples of glands was used on each electrophoresis. The strips of paper were cut into two halves: one half was stained with amidoschwarz and read at a colorimeter, and on the basis of the resulting graph, the corresponding bands of the other half of the paper strip were cut and finally eluted. Twenty-two separations (1100 couples of glands) were run altogether; a typical one is shown on Fig. 1a.

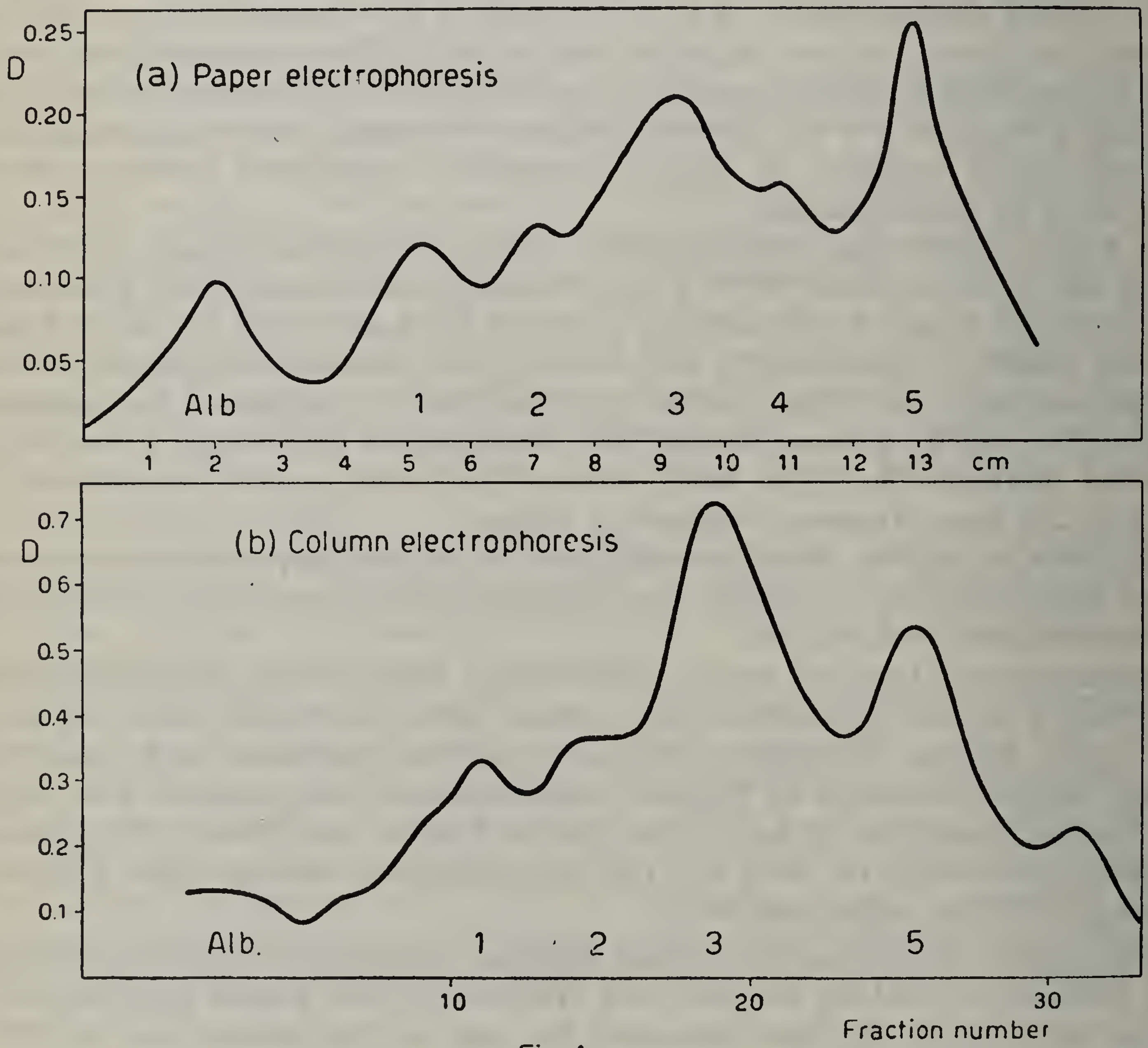

Fig 1

Paper electrophoresis (*a*) and column electrophoresis (*b*) of venomous glands extracts of *Latrodectus tredecimguttatus.*

It was found that fraction no. 1 only shows toxic activity on guinea pig, mouse, rat and *P. americana*. The same component, moreover, shows to be very active on rabbit's isolated ileum and on *Cybister* isolated foregut.

Fraction no. 1 only injected subcutaneously in rabbit induces the same type of hypertension as brought about by the venom *in toto*. This suggests that the

hypertensive factor can be identified with the toxic one, which disagrees with the hypothesis of Troise (43) and Sampayo (40).

The activity of the protein fraction no. 1 appeared, however, to be 10 times lower than that of the protein extract *in toto*, the activity being presumably partly lost during the electrophoretic run or during elution.

Since no hyaluronidase activity in vitro could be evidenced through several trials [McLean and Hale's method (33)] on fraction no. 1, even at concentrations equal to 2.3 g of dry glands/l (the venom *in toto* is active at 0.07 g/l), nor on any of the other protein fractions, the test was repeated parallelly on the total venom as well as on the dialyzed venom: they both showed an equal hyaluronidase activity. It appears, therefore, that the hyaluronidase activity of the venom is not due to a dialyzable substance, but is evidently lost during the electrophoretic run or during elution.

No bronchospastic activity could be evidenced by testing on fraction no. 1, even at concentrations as high as 480 mg of dry glands/l, nor on any of the three other protein fractions, nor on the dialyzate.

From the above reported preliminary work it appears that, for toxicological and pharmacological tests, paper electrophoresis of *Latrodectus* venom presents the disadvantage of yielding only relatively small amounts of fractionated venom available for testing. We have, therefore, deemed useful subjecting considerable amounts of venom to column electrophoresis. One thousand couples of glands, (dry weight 160 mg), were homogenized in the same buffer used for electrophoresis, and centrifuged at 20.000 $\times$ g. The supernatant was employed for electrophoresis on a 2 $\times$ 70 cm cellulose column packed in borate buffer (ionic strength 0.07, pH 8.6), during 26 hrs. (800 V, 28 mA) at a temperature of about 15° C. Elution was carried out collecting fractions of 5.5 ml every 10 minutes, and the density was read at 280 mμ with a D. U. Beckman spectrophotometer. The resulting graph (Fig. 1 b) shows a trend similar to that obtained with paper electrophoresis. The larger protein fractions in the two separating procedures (nos. 1, 2, 3 and 5) evidently correspond in relative quantities and position. The column separation graph, however, shows an extra peak beyond fraction no. 5, while no peak can be observed, in the same graph, corresponding to fraction no. 4 which appears to be well separated in the paper electrophoresis graph.

The various fractions obtained through column separation yielded 16.12 mg of protein [Lowry et al. method (32)] which amounts to about 1/10 of the dry glands weight (160 mg), distributed as follows: fraction no. 1: 9.4%; fraction 2: 16.4 %; fraction 3: 40.0 %; fraction 4: 21.3 %; fraction 5: 7.7 %; fraction 6: 5.1 %.

The LD_{50} for guinea pig on fraction no. 1 was equal to 16 mg of dry glands/Kg. Therefore, the material separated on the column was about 200 fold less active than the total venom. This loss of activity took probably place during the electrophoretic separation since, as we have observed, the total venom in solution looses all of its activity within 3 days at room temperature.

We are planning, for our future work, to carry out further column separations, at low temperature, so as to obtain a large stock of lyophilized toxic protein fraction to be used for further studies.

TABLE I

Toxicity of *Latrodectus tredecimguttatus* Venom *in toto* on Mammals and on Insects.

Animals	LD_{50} values	
	mg*/animal	mg*/Kg
Guinea pig	0.028	0.075
Mouse	0.0135	0.900
Periplaneta americana	0.015	21.0
Musca domestica	0.000013	0.6

* of dry glands used for the extract.

The data reported in the present paper indicate that the same electrophoretic fraction of *Latrodectus* venom is responsible both for its toxicity and for its pharmacological activity on ileum and arterial pressure [1].

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SUMMARY

Extracts of venomous glands of *Latrodectus tredecimguttatus* have been separated electrophoretically in proteic fractions. One only of the proteic fractions has shown to be toxic to vertebrates and insects, and active in some pharmacological tests on vertebrates and insects.

RIASSUNTO

Aspetti biochimici e tossicologici del veleno di Latrodectus tredecimguttatus.

L'estratto di glandole velenifere di *Latrodectus tredecimguttatus* è stato separato elettroforeticamente in frazioni proteiche. Una sola di queste frazioni proteiche è risultata tossica per i Vertebrati e per gli Insetti, ed attiva in alcune prove farmacologiche su Vertebrati e su Insetti.

FRAENKEL G., NAYAR J., NALBANDOV O., YAMAMOTO R. T. (*)

FURTHER INVESTIGATIONS INTO THE CHEMICAL BASIS OF THE INSECT-HOSTPLANT RELATIONSHIP

The role of secondary plant substances as decisive agents in the evolution of the specificity of hostplants for insects has been previously emphasized (Fraenkel 1959 a and b). The general function of glucosides, alkaloids, essential oils, tannins, etc. is to deter insects from feeding on plants. Even polyphagous insects never eat all plants, and their distribution is limited to those which contain no feeding repellents. Oligophagous insects are limited to plants which, in addition, contain feeding attractants. The fact that oligophagous insects are very often limited to several or many species of a particular family of plants makes it very probable that plant families are characterized by certain types of compounds, which then act as the attractants for a particular insect. This is well known in the case of *Cruciferae* and *Umbelliferae* which are characterized by the presence of mustard oil glucosides or essential oils, respectively. In other plant families the occurrence of compounds common to many different species can be inferred from the distribution of insects feeding on them, but little or nothing is known about the nature of such compounds. A research project in operation over a number of years in this department (supported by the Rockefeller Foundation) has been concerned with this chemical aspect of the insect-hostplant relationship. Work to be reported here concerns the specificity of the Tobacco hornworm, *Protoparce sexta*, to plants of the *Solanaceae*; that of the Catalpa worm, *Ceratomia catalpae*, to the Catalpa tree; of the silkworm, *Bombyx mori*, to *Moraceae*; and of the Mexican bean beetle, *Epilachna varivestis* to certain *Leguminosae*.

Various methods are being used in the testing of the activity of fractions prepared from plants during the isolation procedure. The most generally useful method is to present the food in the form of an agar diet, usually containing besides agar, sugar, a protein, and cellulose as a sourse of roughage, into which fractions to be tested are incorporated on a quantitative basis. In the ideal case little or no feeding occurs on the basic diet alone. The amount of feeding

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taking place within a standard period (usually overnight, 12-15 hours) is quantitatively determined by the number of fecal pellets produced. Cases of mere attraction and perhaps biting (sampling) but not feeding are detected by observation of the behavior of the insects and by signs of biting marks left on the surface. A less satisfactory, but sometimes useful method is to apply fractions to be tested on the surface of a leaf commonly eaten to a slight extent so it can be considered free of decidedly repelling substances. Some insects will eat or chew filter paper soaked with suitable preparations.

Protoparce sexta can be raised on many different representatives of *Solanaceae*. On certain plants it begins to eat but soon stops through the presence of seemingly repellent or toxic substances (*Nicandra, Petunia*) (Yamamoto and Fraenkel 1960 b). An attractant principle has been isolated from tomato and potato leaves but not yet fully identified (Yamamoto and Fraenkel 1960 a). It is a glycoside, but certainly not a glycoalkaloid. It is colorless, soluble in water and dilute alcohol while the aglycon is of pungent odor and soluble in fat solvents. The activity is lost after hydrolysis. Neither the glucoside nor the aglycon have so far been crystallized. The glycoside contains C, H, O, and N. Its molecular weight (by ebullioscopic method) was tentatively determined as 405, and the elementary analysis corresponded to $C_{17}H_{29}O_{10}N$. It is assumed that this attractant is widely distributed among *Solanaceae*. The same substance also apparently constitutes the attractant principle for the Colorado Potato beetle, *Leptinotarsa decemlineata*.

This feeding stimulant also must be assumed to be present in *Nicandra* which is attacked but immediately abandoned with signs of distaste. The repellent substance is slightly soluble in water, but is soluble in alcohol, ether, chloroform and other, but not all fat solvents. The molecular weight was approximately 368 and the empirical formula corresponded to $C_{22}H_{27}O$. All chemical and photometric tests pointed to a steroid composition, but it is certainly not a glycoalkaloid. It is also repellent and toxic to the silkworm *Bombyx mori*, and to house flies, but not, for instance to the Armyworm, *Prodenia eridania*.

Petunia is not fed upon naturally by *Protoparce* not so much because of its bad taste but because of some toxic properties. The caterpillars eat the leaves like those of a normal hostplant, but soon symptoms of intoxication such as vomiting, contraction and coma set in. Insects so intoxicated rarely recover. This toxic principle is extracted from the leaves in 90 % methanol and is soluble in many fat solvents, but not in water. It does not contain nitrogen nor sugar, and is neither one of the known toxic solanaceous alkaloids, nor a glycoalkaloid.

The silkworm, *Bombyx mori*, generally regarded as a strictly monophagous insect which feeds only on mulberry, in reality thrives on a number of Moraceae with greater or lesser success (Fraenkel 1959 a and b). The role of specific compounds in mulberry as feeding stimulants has been investigated by several authors (Watanabe 1958, Hamamura 1959). In recent investigations in our laboratory a biting stimulant has been isolated from *Morus* and *Maclura* (another food plant) and identified as a straight chain primary alcohol of a

type known from leaf waxes, with an average composition of $C_{30}H_{62}O$. A compound, obviously identical and also having the same biological effect, has also been isolated from the ethyl ether extracts of several other plants such as the bean, elm and pine. This compound essentially initiates biting responses while continued feeding depends on the presence of other materials in the ether extract. Purification of the ether extract leads to an « oil » which is slightly

TABLE 1

Feeding and biting responses of the 4th instar silkworm larvae to the diets containing various active fractions of the ether extract of dried mulberry leaf powder. Five replicates of one, one - day old, 4th instar larva were used for each test, and the tests were run for 12 hrs.

Diets	No. of larvae responded to diet out of 5 larvae	Total fecal count for 5 larvae	Average fecal count
1. Basic diet (*)	5	19	3.8
2. Basic diet + 4 % Mulberry leaf powder	5	63	12.6
3. Basic diet + Unsaponifiable, phase-separated fraction of Ether extract of Mulberry leaf powder (2 mg/1 ml of diet)	5	57	11.4
4. Basic diet + EtOH soluble fraction of No. 3 (Oily in nature) (2 mg/ml of diet)	3	55	18.3
5. Basic diet + Purified long-chain Primary alcohols separated from fraction No. 3 (1 mg/ml of diet)	5	27	5.4
6. Basic diet + Fractions from No. 4 and 5 above	5	70	14.0
7. Basic diet + water ext. of Ether extracted Mulberry leaf powder (15 mg/ml of diet)	4	72	18.0

(*) Basic diet consists of: Agar agar 4 gm; Cellulose 4 gm; Casein 1 gm; Extracted Tomato Leaf Powder, 2 gm; Sucrose 4 gm; Yeast 0.5 gm; Ascorbic acid 0.1 gm; water, 100 ml.

colored and which is very active in stimulating continued feeding. An artificial diet consisting of casein, sucrose, cellulose, yeast and agar is fed upon slightly in the presence of the « alcohol », and is fed upon for a longer period of time upon the addition of the « oil » (Table 1). On the other hand, there also appears to be a stimulating substance present in the residue of the ether extract and which can be extracted with ethanol or water. (The stimulating activity

of the aqueous extract appears to be present only in spring collected leaves for leaves collected during the summer do not show any activity). However, aqueous extracts of elm, bean, or pine leaves do not stimulate feeding but actually inhibit feeding. The biting stimulant therefore appears to be present in a wide number of plants whereas the feeding stimulant, extractable with ethyl ether, appears to be restricted to *Moraceae.*

The Catalpaworm, *Ceratomia catalpae (Sphingidae)*, occurs profusely on leaves of the Catalpa tree, *Catalpa bignonoides*, in the United States, and is not known from any other plant besides *Catalpa.* We have tested it on several other representatives of the same family, *Bignoniaceae*, with negative results. A fraction, active at 2 mg/ml, has been prepared from *Catalpa* leaves by extraction with 95 % alcohol and treatment with lead acetate and magnesium oxide. It contains a glucoside and loses its activity on hydrolysis (Table 2). This procedure of purification follows the first steps in the isolation of catalposides, as described by Collin *et al.* (1943) and Chollet (1959). The possibility of catalposides constituting the active attractant principle in the feeding of *Ceratomia* is being investigated.

The Mexican bean beetle, *Epilachna varivestis*, feeds on many representatives of *Leguminosae*, but is largely restricted to species of the genus *Phaseolus* (Lippold 1957). Alcoholic extracts from bean leaves are a powerful stimulant

TABLE 2

Feeding response of the larvae of *Ceratomia catalpae* to diets containing various extracts of Catalpa leaf powder. Five replicates of two, newly molted, 3rd instar larvae were used per test, and tests were run for a 24 hr. period.

Diets used	Total fecal count of 10 larvae	Average fecal count per larvae
1. Basic diet (*)	98	9.8
2. Basic diet + EtOH extract of Catalpa leaf powder (200 mg/100 ml diet)	328	32.8
3. Basic diet + supernatant from Pb acetate treatment of No. 2 extract after the method of Collin et al. 1943 (160 mg/100 ml of diet)	336	33.6
4. Basic diet + No. 3 extract treated with MgO and re-extracted with EtOH (100 mg/100 ml of diet)	324	32.4
5. Basic diet + No. 4 extract hydrolyzed with 5 % H_2SO_4 (100 mg/100 ml of diet)	20	2.0

All extracts were equivalent to 4 % of Catalpa leaf powder.

(*) Basic diet consists of: agar 4 gm, cellulose 4 gm, glucose 2 gm, casein 1 gm, yeast 0,5 gm, and water 100 ml.

for feeding. These extracts were further concentrated and purified by treatment with lead acetate, chromatography on alumina and decoloration with charcoal. Such extracts from bean leaves would be expected to contain the cyanogenetic glucoside linamarin. Linamarin itself has not yet been tested for its role in stimulating the bean beetle to feed. A preparation of glucosides isolated from white clover and stated to contain linamarin and lotaustralin in about equal amounts (*) did not show a clear-cut activity over and above that obtained with glucose alone, but the possibility still exists that linamarin alone tested under suitable conditions might reveal attractant properties.

(*) We are much indebted to Dr. G. W. Butler, D.S.I.R., Palmerston North, New Zealand, for this preparation.

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SUMMARY

Work is being reported in the isolation and characterization of compounds from plants which stimulate or prevent feeding by particular insects. This concerns the specificity of the Tobacco hornworm, *Protoparce sexta*, to plants of *Solanaceae*, that of the silkworm, *Bombyx mori*, to *Moraceae*, of the Catalpa worm, *Ceratomia catalpae*, to the Catalpa tree and of the Mexican been beetle, *Epilachna varivestis*, to certain *Leguminosae*.

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RIASSUNTO

Ulteriori ricerche sulle basi chimiche delle relazioni fra Insetti e piante ospiti.

E' riportato un lavoro sull'isolamento e caratterizzazione di composti di origine vegetale che stimolano o inibiscono l'alimentazione di particolari Insetti. Questo lavoro concerne la specificità di *Protoparce sexta* per le *Solanaceae*, di *Bombyx mori* per le *Moraceae*, di *Ceratomia catalpae* per *Catalpa*, di *Epilachna varivestis* per certe *Leguminosae*.

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THE PHYSIOLOGICAL BASIS FOR THE SELECTION OF PLANTS FOR EGG-LAYING IN THE TOBACCO HORNWORM, *PROTOPARCE SEXTA* (JOHAN.)

In a previous communication (Yamamoto and Fraenkel 1960 a), the specificity of the moths of the tobacco hornworm to solanaceous plants was reported. Under laboratory conditions oviposition occurred only on solanaceus plants, even on plants such as *Petunia hybrida* Vilm. or *Nicandra physalodes* (L.), which were found to be toxic and repellent respectively to the larvae. On the other hand, in preference tests in which the moths were exposed to two species of solanaceous plants simultaneously, the moths significantly preferred to oviposit on tomato, *Lycopersicon esculentum* Mill., over the other plants. Field surveys in Champaign county, Illinois, also disclosed that of the more than seventeen species of solanaceous plants available coincidentally with the appearance of the moths during the summer months, only three species of plants, tomato and two species of tobacco, were naturally infested. Of these, tomato was predominantly and apparently preferentially infested.

These experiments in the laboratory and observations in the field suggested that within a broad base of plants which can support the larvae normally, the moths limited their choice of plants by means of recognition cues. It was further suggested that two distinct and perhaps separable stimuli might be involved in the manifestation of selection and oviposition: 1) a stimulus which triggers oviposition and termed the « oviposition stimulus » and which is apparently present in all the solanaceous plants tested, and 2) a stimulus, possibly discrete and peculiar to each species of plant and to which the insects become habituated through constant contact during the larval stages. This latter stimulus was termed the « orientation stimulus ». Investigations conducted along this line since then give support to the contention that two chemostimuli are involved in the host plant selection of the tobacco hornworm moths. These recent findings are reported here.

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MATERIAL AND METHODS

The moths used in the experiments were reared in the laboratory. All fresh plants and plant materials were either cultivated in greenhouses or gathered in the field.

In the communication mentioned previously, the moths used in the preference tests were from larvae reared exclusively on tomato. To test whether the preferences of the moths for tomato might have been influenced by the diets of the larvae, three other species of solanaceous plants were used to rear the larvae. These plants were the potato (*Solanum tuberosum* L.), purple nightshade (*Solanum dulcamara* L.) and matrimony-vine (*Lycium halimifolium* L.). These plants were chosen either because of their natural abundance or ease in culti-

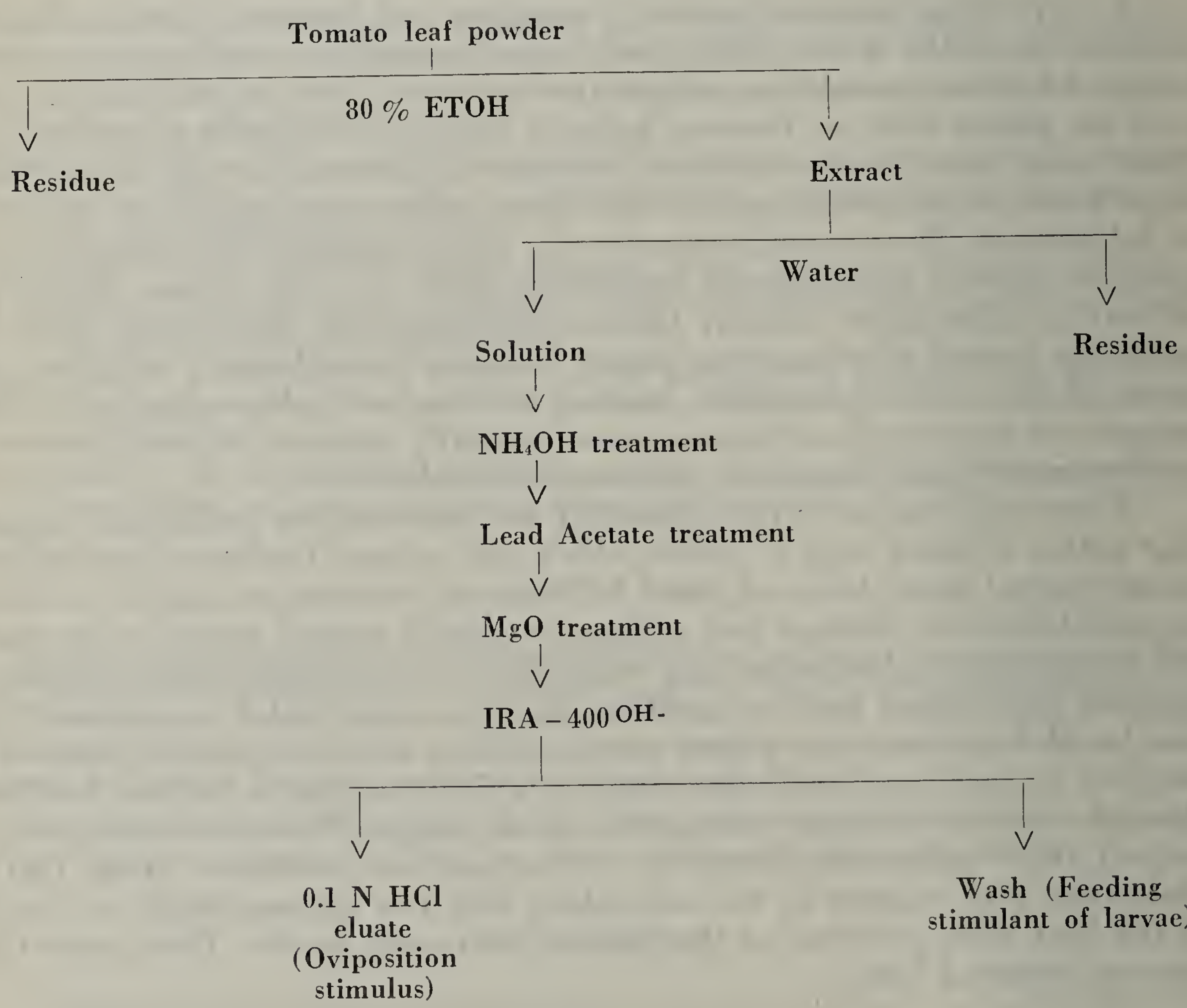

Fig. 1.

Schematic outline showing the treatment used in the purification of the « oviposition stimulus ». The « oviposition stimulus » is separated from the feeding stimulant of the larvae by use of a basic ion exchange resin, IRA – 400 OH^-.

vation. The moths, reared during the larval stages on these plants, were subsequently assayed for their preferences in oviposition in a cage 3' × 5' × 3' under two-choice situations. Tomato was used as the control plant in each case. The plants were represented by freshly cut foliages placed in quart mason jars and the jars placed four feet apart on opposite longitudinal ends of the cage. They were exposed to gravid moths overnight in a dimly lit room. The number of eggs deposited on the plants was used as the criterion of preference.

Dried tomato leaves were pulverized and extracted with 80 % ethanol in a soxhlet extraction apparatus. This crude ethanol extract, containing the « oviposition stimulus », was further purified and assayed (figure 1). Crude extracts of other solanaceous and non-solanaceous plants were assayed for their activities only, and further chemical treatments were not applied on them. The odoriferous components of fresh tomato leaves were obtained by simple steam distillation. The various extracts were assayed by spraying them, in aqueous solutions, on bean plants (*Phaseolus* sp., *Leguminosae*) (Table 1) and on « dummy » plants

Table 1.

The stimulatory effect of various extracts of tomato leaves in inducing oviposition in the moths of the Tobacco hornworm. The extracts were sprayed on a non-host plant, bean, in aqueous solution.

Preparation	Trials	Moths	Total Eggs
Alcohol extract, crude	4	20	518
Alcohol extract, purified up to MgO treatment (*)	2	5	392
Ether extract, crude	1	5	0
Steam distillate	2	15	39
Water (control)	1	5	0

(*) See fig. 1.

constructed of wire and cloth (Table 2, figure 2). The plants so treated with the extracts were then exposed to the moths overnight.

The larvae, while being reared on the various species of plants, were also subjected to preference tests during the 2nd, 3rd, and 4th instars. These preference tests were carried out to reveal whether habituation to unknown factors were being established incipiently in them. For these tests, 10 cm. diameter petri dishes with the appropriate size filter papers were employed. The filter papers were sectioned into four equal parts, and to each section, a 1.5 cm diameter disc from a leaf was placed. One larva, starved for two hours before

the experiment, was then placed in the center of each dish and allowed to wander and feed on the discs. The leaf disc initially sampled and the subsequent continued feeding on a disc after various time intervals were recorded. Twelve replicates were used in these tests.

Fig. 2.
Photograph of a «dummy» plant employed in the assay of the «oviposition stimulus» from crude extracts of various plants. The «dummy» plant was constructed of wire screen covered with heavy gauge cloth. The extracts were sprayed on the «dummy» plants just prior to exposing them to moths kept in a large breeding cage.

RESULTS AND DISCUSSION

The presence of a stimulatory material which induces oviposition and which is extractable from fresh or dried leaves of the tomato is shown in Table 1. This material is soluble in water and dilute ethanol but insoluble in organic solvents such as ether, acetone, and chloroform employed during preliminary extractions. It is not steam distillable and does not appear to be an odoriferous

or volatile component of the plant. From observations of the moths in the act of oviposition, the material is apparently perceived by contact since the moths oviposited only in the areas of the « dummy » plants which had been sprayed with the extract. The steam distillable fraction, on the other hand, although inactive in inducing oviposition, appears to attract the moth to the plants on which it is applied. Thus when the steam distillate is sprayed in combination with the ethanol extract, the two together induced the deposition of nearly three times as many eggs as the ethanol extract alone (Table 2). It appears that the two stimuli act in sequence, the steam distillable component attracting the moths to the plant and the ethanol extractable component then inducing the moths to oviposit.

TABLE 2.

Ovipositional response of the moths of the Tobacco hornworm to crude extracts of tomato leaves sprayed on « dummy » plants constructed of cloth covered screen wires. The tests were conducted unter two-choice preference situation. Seven gravid moths were used for each set of experiments.

Experiment	Preparation	Total Eggs
1	Steam distillate	26
	Alcohol extract	281
2	Steam distillate + alcohol extract	481
	Alcohol extract	170

The chemical identity of the « oviposition stimulus » is not known as yet. Purification by various chemical treatments (fig. 1) indicate that this material is not identical with the feeding stimulus of the larvae which was previously extracted from dried tomato leaves and tentatively identified as a glycoside (Yamamoto and Fraenkel 1960 b). Assays of crude extracts of many plants further revealed that all the solanaceous plants as a group contain a substance which induces oviposition. In contrast, extracts of non-solanaceous plants were inactive.

Larval habituation to plants used in the experiments was easily demonstrable. Larvae which were exposed to a choice of leaf discs from four species of plants preferred to feed on the discs of the plant on which they had fed previously (Table 3). This preference for a particular plant was already displayed in the 2nd instar larvae, and in all cases, by the 3rd instar, the majority of the larvae showed this preference. It would appear that each plant species is sufficiently different chemically so that the larvae can recognize these differences after a period of feeding. Assumptions that larval habituation to a single plant would be carried through to the moths, however, were not readily borne out. Moths reared during the larval stages on *Lycium* or *S. dulcamara* still pre-

ferred to oviposit on tomato (Table 4). Only in the case of potato, *S. tuberosum*, where the distribution of the eggs was nearly equal on that plant as on tomato, was there any indication that prior habituation during the larval stages might be reflected in the choice of plant by the moths. When offered a choice bet-

TABLE 3.

The feeding preferences of the larvae of the Tobacco hornworm reared on four species of plants. The larvae were reared on the plants up to the instars indicated below and then exposed to 4-choice preference situations. Twelve larvae, confined individually, were used for each tests. Duration of the tests was 2 hours.

Plant larvae were reared on	Larval instar	Preferred plant (a)
Tomato	2nd	Tomato
	3rd	»
	4th	»
Solanum dulcamara	2nd	S. dulcamara
	3rd	»
	4th	»
Solanum tuberosum	2nd	S. tuberosum
	3rd	»
	4th	»
Lycium halimifolium	2nd	none
	3rd	L. halimifolium
	4th	»

(a) The preferred plant is listed when 50% of more of the experimental insects responded positively to the plants on which they had fed previously.

TABLE 4.

The ovipositional preferences of moths of the tobacco hornworm reared during the larval stages on four species of solanaceous plants. The moths were exposed to the plants listed below under two-choice situations for 12 hours.

Plant larvae were reared on	Number of moths	Percent distribution of eggs on plants		Total eggs
Tomato	6	Tomato 69.5	S. tuberosum 30.5	760
Solanum tuberosum	8	Tomato 51.3	S. tuberosum 49.7	2672
Tomato	6	Tomato 72.4	S. dulcamara 27.6	1223
Solanum dulcamara	6	Tomato 64.1	S. dulcamara 35.9	1697
Tomato	7	Tomato 81.2	L. halimifolium 18.8	860
Lycium halimifolium	3	Tomato 83.8	L. halimifolium 16.2	204

ween potato and tomato, about 30 % of the eggs of moths raised on tomato were laid on potato, against 50 % when they had been raised on potato. Since the experiments were undertaken for only one generation, the possibility remains, therefore, that with some of the plants a longer period of habituation through several generations might be necessary for shifts in the plant preferences of the moths to occur.

CONCLUSIONS

The specificity of the moths of the tobacco hornworm to solanaceous plants can be attributed to specific chemical stimuli. The chemicals affording the stimulation necessary for oviposition apparently occur only in solanaceous plants and the response to these chemicals may be considered to be part of the genetic constitution of the moths. On the other hand, preferences for only a few individual plants within the family Solanaceae appear to be responses induced by habituation to other chemical factors present only in specific plants.

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SUMMARY

(see « Conclusions »)

RIASSUNTO

Le basi fisiologiche della scelta delle piante per la deposizione delle uova nel Lepidottero Protoparce sexta *(Johan).*

La specificità del Lepidottero *Protoparce sexta* verso Solanacee può essere attribuita a stimoli chimici specifici. Prodotti chimici che permettono la stimolazione necessaria per l'ovoposizione sono presenti apparentemente solo nelle Solanacee ed il responso a questi prodotti chimici può essere considerato parte della costituzione genetica del Lepidottero. D'altra parte le preferenze solo verso alcune piante della famiglia delle Solanacee sembrano essere indotte per abitudine verso altri fattori chimici presenti solo in esse.

Auclair J. L. (*)

TENEUR COMPAREE EN COMPOSES AMINES LIBRES DE L' HEMOLYMPHE ET DU MIELLAT DU PUCERON DU POIS, *ACYRTHOSIPHON PISUM* (HARR.), A DIFFERENTS STADES DE DEVELOPPEMENT

Les acides aminés occupent une place d'intérêt primordial dans l'étude du chimisme de l'être vivant. Une vingtaine de ces substances existent sous forme de métabolites aux niveaux cellulaire et humoral, mais se rencontrent surtout comme constituants fondamentaux des protéines. Une des principales caractéristiques biochimiques des insectes est la forte teneur de leur milieu intérieur en composés aminés. Ce fait a été reconnu dès le début du siècle par les travaux de Nazari en 1902 (Florkin 1944), suivis de ceux de Bishop, Briggs et Ronzani (1925), Duval, Portier et Courtois (1928), Heller et Moklowska (1930), Florkin (1937), Babers (1938) et plusieurs autres. D'après ces travaux, la teneur totale en azote aminé de l'hémolymphe de plusieurs espèces de Coléoptères, Lépidoptères et Hyménoptères était comprise généralement entre 40 et 350 mg par 100 ml. Depuis, les méthodes modernes de dosage microbiologique et surtout l'avènement de la méthode de chromatographie de partage sur papier filtre en 1944 ont permis l'identification des composés aminés de l'hémolymphe par de nombreux auteurs (e.g. Raper et Shaw 1948; Drilhon et Busnel 1949; Finlayson et Hamer 1949; Agrell 1949; Levenbook 1949; Drilhon 1950; Auclair et Patton 1950; Pratt 1950; Drilhon, Busnel et Vago 1951; Auclair et Dubreuil 1952 et 1953; Chen et Hadorn 1954). Roeder (1953) et Duchâteau et Florkin (1958) ont publié d'excellentes revues sur le sujet, et une autre par Wyatt (1961) est actuellement en préparation.

Les composés aminés libres de l'hémolymphe et des excrétions des insectes suceurs phytophages (Hémiptères et Homoptères) ont été cependant très peu étudiés. Pratt (1950) a rapporté 13 acides aminés dans le sang d'*Oncopeltus*

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fasciatus. Dans le miellat excrété par les aphides et les coccides, des quantités assez étonnantes de ces composés ont été observées par Auclair et Maltais (1952), Maltais et Auclair (1952), Gray (1952), Lamb (1953 et 1959), Mittler (1953 et 1958), Ewart et Metcalf (1956) et Auclair (1958).

Le présent travail traite de l'identification et de l'analyse semiquantitative des composés aminés libres de l'hémolymphe et du miellat du puceron du pois, *Acyrthosiphon pisum* (Harr.), au quatrième stade larvaire et au stade adulte avant et pendant la reproduction, ainsi que de l'influence de certains facteurs sur la concentration de ces substances dans ces deux milieux biologiques.

METHODES EXPERIMENTALES

Une population de pucerons virginipares aptères de la race Rl (Cartier 1957) était maintenue constamment en serre. La culture des plants de pois (*Pisum sativum L.*, variété Perfection) en serre et sous des conditions déterminées a déjà été décrite (Auclair 1958). Toutefois, pour les fins de cette étude, les plants étaient cultivés dans des pots de 12 pouces de diamètre, et disposés en une rangée centrale d'une vingtaine de plants par pot. Lorsque les plants avaient 4 à 6 semaines d'âge, un carton d'environ 8 par 16 pouces et recouvert d'un papier ciré était placé horizontalement de chaque côté du rang au niveau du quatrième entrenoeud des plants, la pousse terminale représentant le premier entrenoeud. Les plants étaient alors infestés de pucerons d'âge uniforme, (i.e. nés dans un même intervalle de 24 hr), à raison de 10 à 20 pucerons par plant, et le papier ciré recouvert de papier filtre. Lors de la collection du miellat, le papier filtre était enlevé et les gouttelettes tombant sur la surface cirée étaient prélevées immédiatement à l'aide d'une micropipette capillaire de 5 microlitres subdivisée au 0.5 de microlitre. Des volumes déterminés de ce miellat frais étaient ensuite déposés sur des feuilles de papier filtre taillées en vue de la séparation bidimensionnelle chromatographique. Des séries de chromatogrammes de 0.5, 1, 2 et 4 microlitres de miellat étaient préparées en *quadruplicata*, avec en plus quelques chromatogrammes de 8, 16 ou même 20 microlitres.

La saignée des pucerons suivait immédiatement la collection du miellat et s'opérait comme suit: les pucerons étaient submergés pendant 2 minutes dans de l'eau à 62° C. Ce traitement avait pour but d'inactiver les enzymes et d'empêcher ainsi la coagulation et la mélanose du sang. Chaque puceron était ensuite séché extérieurement sur du papier filtre, les antennes sectionnées à la base et un échantillon de sang variant de 0.1 à 0.2 microlitre prélevé avec la micropipette ayant servi à la collection du miellat. L'opération s'effectuait à l'aide de micro-ciseaux et sous une loupe binoculaire (grossissement de 6 à 10 fois). Des volumes d'hémolymphe identiques à ceux mentionnés ci-dessus pour le miellat étaient chromatographiés simultanément. Dans d'autres expériences effectuées en même temps que celles décrites ci-dessus, des plants plus âgés (i.e. 7 à 8 semaines) étaient infestés à raison de 10 à 20 pucerons par plant dans un cas, et des plants de 4 à 6 semaines étaient infestés sévèrement, à raison de 50

à 100 pucerons par plant dans l'autre cas. Les résultats sur l'hémolymphe et le miellat étaient comparés dans toutes ces expériences. D'autres analyses ont aussi été effectuées sur le plasma, les cellules sanguines ayant préalablement été enlevées par ultracentrifugation. La technique de chromatographie quantitative utilisée dans ce travail a déjà été décrite (Auclair et Maltais 1954; Auclair, Maltais et Cartier 1957).

RESULTATS

Les résultats des analyses ont révélé que l'hémolymphe et le miellat possèdent une teneur totale élevée sensiblement équivalente en composés aminés libres, laquelle varie de 1000 à 3000 microgrammes par 100 microlitres. Quatorze à seize composés se rencontrent généralement dans ces deux milieux. Ce sont les acides aspartique et glutamique et les amides correspondants asparagine et glutamine, la glycine, l'homosérine et la sérine, la leucine et/ou l'isoleucine, la méthionine, la phénylalanine, la proline, la thréonine, la tyrosine, la valine et probablement l'arginine. L'alpha-alanine, la cystine et l'acide alpha-amino-butyrique ne sont présents généralement que dans le sang, tandis que l'acide gamma-amino-butyrique n'apparaît ordinairement que dans le miellat. De plus, aucune différence qualitative dans les composés aminés n'a été observée entre le plasma sanguin et l'hémolymphe, mais, dans certaines analyses, une légère différence quantitative en faveur du plasma a pu être décelée.

Dans l'histogramme (Fig. 1) sont indiquées les concentrations relatives de chaque composé aminé du miellat et de l'hémolymphe de puceron adulte juste avant la période de reproduction. Le sang est ordinairement plus riche que le miellat en cystine, alanine, proline, tyrosine, valine et leucines, tandis que ce dernier est plus riche en autres composés. L'acide glutamique fait ici exception, car il prédomine ordinairement dans le miellat. Dans le cas présent (Fig. 1), si l'on exclut l'homosérine, la teneur totale en composés aminés du miellat et de l'hémolymphe est sensiblement la même, soit 1437 contre 1425 microgrammes par 100 microlitres.

Plusieurs facteurs peuvent faire varier la teneur totale en composés aminés libres. Chez la larve au quatrième stade, le miellat est ordinairement plus riche que le sang en composés aminés, tandis que chez le jeune adulte, avant la période de reproduction, ces deux milieux s'équilibrent (Fig. 1). Néanmoins, durant la période de reproduction, la teneur du miellat en ces composés tend à s'abaisser sensiblement, par rapport au sang. Cette diminution dans le miellat peut résulter d'un besoin accru d'azote chez la femelle en parturition, et d'un appauvrissement progressif de la sève de l'hôte parasité qui doit nourrir un nombre toujours croissant de pucerons. D'autres expériences ont démontré que lorsque les plants utilisés sont plus âgés (i.e. 7 à 8 semaines d'âge), ou lorsque l'infestation des plants de 4 à 6 semaines est accrue à 50-100 pucerons par plant, la teneur totale en acides aminés du miellat et de l'hémolymphe peut s'abaisser de 10 à 50 % de sa valeur originale.

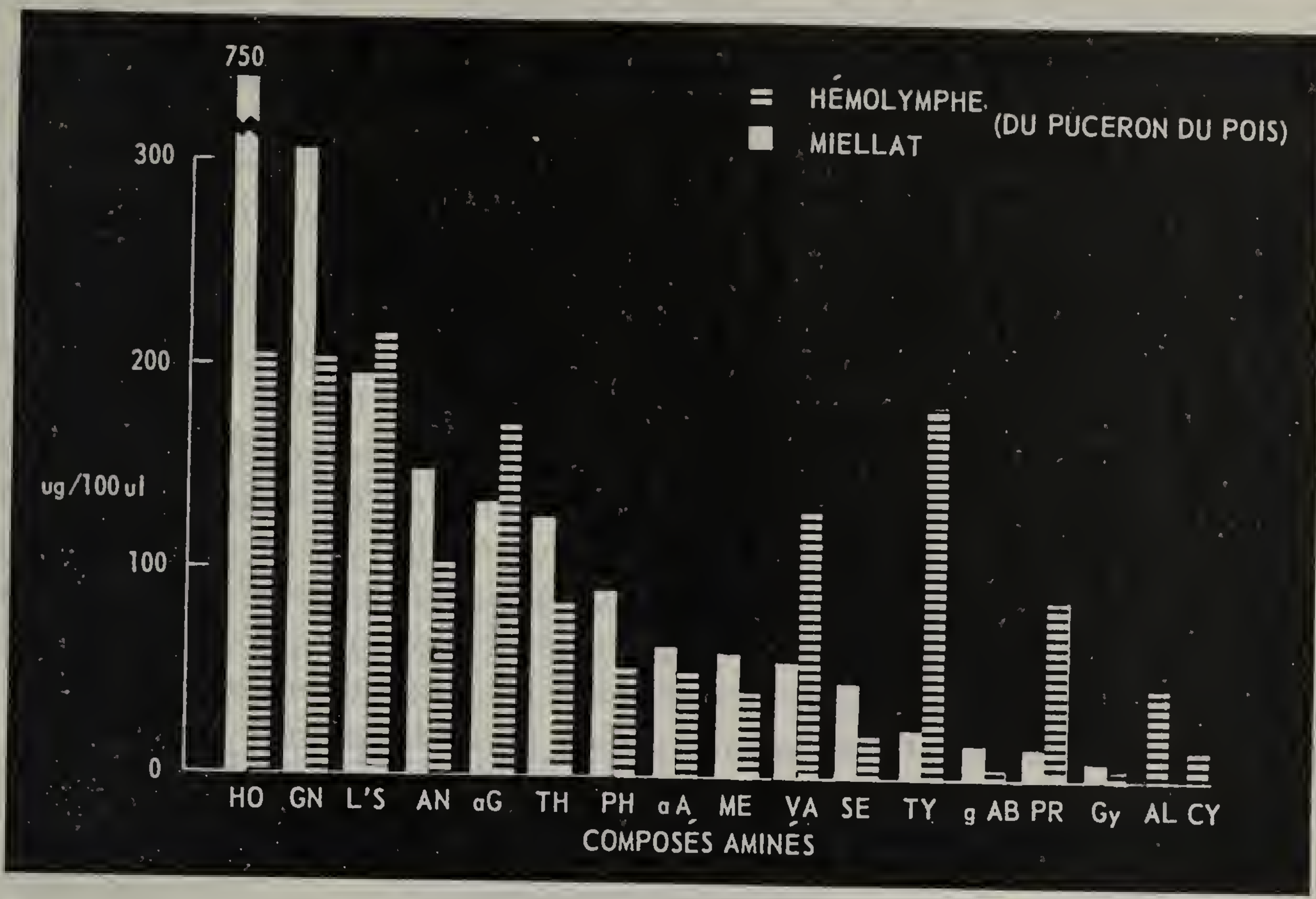

Fig. 1

Teneur comparée (microgrammes par 100 microlitres) en composés aminés libres du miellat et de l'hémolymphe du puceron du pois, *Acyrthosiphon pisum* (Harr.), au stade adulte virginipare aptère avant la période de reproduction. Les pucerons étaient maintenus sur des plants de pois (*Pisum sativum* L., variété Perfection) âgés de 4 à 6 semaines, à raison de 10 à 20 pucerons par plant. HO, homosérine; GN, glutamine; L'S, leucine et/ou isoleucine; AN, asparagine; aG, acide glutamique; TH, threonine; PH, phénylalanine; aA, acide aspartique; ME, méthionine; VA, valine; SE, sérine; TY, tyrosine; gAB, acide gamma-amino-butyrique; PR, proline; GY, glycine; AL, alanine; CY, cystine. La teneur totale en composés aminés par 100 microlitres est de 2187 microgrammes pour le miellat et de 1630 microgrammes pour l'hémolymphe.

Les légères différences qualitatives et quantitatives observées pour certains acides aminés entre le miellat et l'hémolymphe peuvent résulter en partie d'une absorption sélective chez le puceron, puisque tous ces composés se retrouvent également chez l'hôte (Auclair, Maltais et Cartier 1957).

La présence dans le miellat de tous ces composés aminés en concentration aussi élevée et comparable à celle de l'hémolymphe demeure difficile à expliquer. L'excrétion de ces substances d'importance fondamentale dans la constitution du protoplasme indiquerait d'une part un métabolisme intermédiaire très peu efficace, découlant peut-être de la vie parasitaire très spécialisée des pucerons, ou, d'autre part, d'une surabondance de ces substances dans la diète.

Certains auteurs (cités par Ewart et Metcalf 1956) ont déjà énoncé l'hypothèse que l'excrétion abondante de matières sucrées chez les aphides résultait d'une carence des aliments en matières azotées. Ils ont supposé que les aphides afin d'obtenir suffisamment d'azote, devaient ingérer de l'eau et des sucres en excès, pour ensuite excréter ces derniers sous forme de miellat via la chambre filtrante. Cependant, l'absence de chambre filtrante chez le puceron du pois, de même que l'excrétion abondante d'acides aminés dans leur miellat rendent, dans le cas présent, cette hypothèse non fondée. Il est probable que la sève absorbée par le puceron du pois contient une teneur élevée en acides aminés libres, tel que démontré déjà par Lindemann (1948) et Mittler (1953) chez d'autres pucerons, et qu'il s'établit un équilibre entre la sève ingérée et l'hémolymphe. Si la sève s'appauvrit en acides aminés, comme par exemple lorsque l'hôte vieillit ou qu'il nourrit un trop grand nombre de parasites, la teneur en azote aminé de l'hémolymphe et du miellat tend à baisser. Si au contraire, la sève ingérée est riche en ces composés (e.g. chez le jeune plant supportant peu de pucerons) l'hémolymphe et le meillat peuvent s'en enrichir considérablement. Ceci expliquerait certaines des variations mentionnées précédemment.

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SUMMARY

It is a well-known fact that the hemolymph of insects is high in free amino acid content. Yet, these compounds are rarely excreted in large quantities. Quantitative analyses by paper chromatography of the hemolymph and honeydew of the pea aphid *Acyrthosiphon pisum* (Harr.), have shown the total free amino acid content to be high and about similar in both fluids, and ranging from 1000 to 3000 micrograms per 100 microliters. Aspartic and glutamic acids and the amides asparagine and glutamine, glycine, homoserine, serine, leucine (and/or isoleucine), methionine, phenylalanine, proline, threonine, tyrosine, valine and probably arginine are present in both media. Alpha-alanine, cystine and alpha-amino-butyric acid are usually observed only in hemolymph, whereas gamma-amino-butyric acid generally appears only in honeydew. The concentration of these compounds may vary greatly especially in honeydew, and may be influenced in part by the stage of development of the aphid and its host, and by aphid population density per plant.

RIASSUNTO

Tenore comparato in composti aminici liberi dell'emolinfa e della melata dell'Afide Acyrthosiphon pisum *(Harr.) a differenti stadi di sviluppo.*

E' un fatto ben noto che l'emolinfa degli Insetti ha un alto contenuto in aminoacidi liberi. Eppure questi componenti sono raramente espulsi in grandi quantità. Analisi quantitative per mezzo di cromatografia su carta di emolinfa e melata dell'Afide *Acyrthosiphon pisum* (Harr.), hanno mostrato che il contenuto totale in aminoacidi liberi è alto e circa simile in entrambi i liquidi, e distribuito da 1000 a 3000 microgrammi per 100 microlitri. Gli acidi aspartico e glutammico e gli amidi asparagina e glutammina, glicina, omoserina, serina, leucina (e/o isoleucina), metionina, fenilalanina, prolina, treonina, tirosina, valina e probabilmente arginina sono presenti in entrambi i liquidi. Alfa-alanina, cistina e acido alfa-amino-butirrico sono di solito presenti solo nell'emolinfa, mentre l'acido gamma-amino-butirrico generalmente appare solo nella melata. La concentrazione di questi componenti può variare grandemente specialmente nella melata e può essere influenzato in parte dallo stadio di sviluppo dell'Afide e del suo ospite, e dalla densità di popolazione degli Afidi per pianta.

Kloft W. (*)

NACHWEIS FREIER AMINOSÄUREN ALS PHYTOPATHOLOGISCH WIRKSAME STOFFE IM SPEICHEL PLANTISUGER INSEKTEN

Im Rahmen unserer Untersuchungen über die Wechselbeziehungen zwischen pflanzensaugenden Insekten und dem von ihnen besogenen Wirtspflanzengewebe (Kloft, 1956-60; Kloft, Ehrhardt, 1959) galt unser besonderes Interesse der chemischen Zusammensetzung des Speichels der bearbeiteten Rhynchoten. Diese Insekten geben bekanntlich im Speichelrohr ihres als Doppelkapillare ausgebildeten Rüssels eine Flüssigkeit ab, die im Zusammenhang mit dem Einstich und der Nahrungsaufnahme in die Pflanze injiziert wird. Neben mechanischen Effekten ist vor allem der Speichel für die Auslösung physiologisch und morphologisch fassbarer Effekte im Wirtspflanzengewebe verantwortlich. Untersuchungen von Steinberg 1949, sowie Commoner, Nehari 1953, hatten gezeigt, dass sich bei pflanzlichen Viruserkrankungen qualitative Verschiebungen des Gehaltes an freien Aminosäuren (AS) einstellen und experimentelle Arbeiten hierzu zeigten, dass sich durch Einbringen dieser AS in Pflanzen die Symptome einer Virose auch ohne Infektion herbeiführen lassen. Nachdem zahlreiche durch Virosen hervorgerufene Krankheitserscheinungen den durch Pflanzenläuse (vermutlich auch ohne Virusbeteiligung) im Stichbereich erzeugten pflanzlichen Reaktionen gleichen, vermuteten wir im Speichel pflanzensaugender Insekten freie Aminosäuren, deren Nachweis mit papierchromatographischen Methoden angegangen wurde.

Bei Heteropteren konnte der Speichel direkt durch Punktierung des Reservoirs der präparatorisch leicht zugänglichen Hauptspeicheldrüse gewonnen werden. Es liess sich ein ganzes Spektrum freier Aminosäuren im Speichel nachweisen, bei Pyrrhocoris apterus L. 13, bei Palomena prasina L. 7 AS, zwischen diesen Wanzenarten bestehen neben qualitativen auch quantitative Unterschiede im AS-Gehalt. (Die Auswertung erfolgte semiquantitativ nach der Fleckenvergleichsmethode).

Wesentlich grössere Schwierigkeiten macht die Gewinnung reinen Speichels bei phloemsaugenden Aphiden, deren Speicheldrüsenreservoir wegen

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seiner Kleinheit nicht punktiert werden kann. Im Grünlicht liessen sich jedoch direkt Anstiche in befeuchtetes Chromatographiepapier auslösen, der hier deponierte Speichel konnte entweder eluiert und dadurch konzentriert oder direkt entwickelt werden. Störungen durch AS des Honigtaues wurden sorgfältig vermieden. Die Verwendbarkeit unserer Methode konnte jetzt von Schäller (1960) auch an Rebläusen bestätigt werden, sie gewährleiste t die Analyse reinen Speichelsekretes, während bei dem von Anders (1957, 1958) vorgeschlagenen Verfahren, bei welchem auf dem Rücken liegenden Rebläusen herausquellender Speichel abgenommen wird, eine Regurgitation von Darminhalt nicht ausgeschlossen werden kann. Im übrigen ist die Methode von Anders ohnehin nur bei Parenchymsaugern anwendbar.

Bei der Aphidide *Myzus ascalonicus* Donc., einem unserer Standardversuchstiere, konnten 4 freie AS (Glutaminsre., Glykokoll, Alanin, Asparaginsre.), bei der bes. an Sitkafichte schädlichen Aphidide *Elatobium abietinum* (Walk.) 7 freie AS (Alanin, l-Arginin, l-Asparaginsre., Cystin, l-Leucin, Serin und Valin) gefunden werden, mit weiteren AS ist prinzipiell jeweils zu rechnen. Sie dürften jedoch wegen ihrer zu geringen Konzentration bisher nicht fassbar gewesen sein (eine Speichelinjektion dürfte sich bei einer Aphidide auf grössenordnungsmässig 10^{-4} mm^3 belaufen, ca. 200 Anstiche wurden gemeinsam auf dem Chromatogramm zweidimensional [Phenol-Collidin] entwickelt).

Die Anwesenheit freier AS im Speichel plantisuger Insekten ist nicht verwunderlich, denn — das zeigte auch das vorausgegangene Referat von Auclair (1960) — in der Haemolymphe der Blattläuse und allgemein der Insekten sind freie AS in relativ sehr hohen Konzentrationen vorhanden. Nach unseren mit P^{32} durchgeführten Untersuchungen scheidet die Speicheldrüse der Aphididen nach Art eines Exkretionsorganes in hoher Konzentration in der Haemolymphe enthaltene niedermolekulare Stoffe sehr leicht und rasch aus (Kloft, Kunkel, 1960). Auch mit dem Gift von Ameisen werden nach Osman, Brander (i. Druck) beträchtliche Mengen freier AS ausgeschieden.

Es sei hervorgehoben, dass die ausgeschiedenen freien AS des Speichels, wie wir das dereits 1956 erstmalig publizierten, phytopathologische Bedeutung besitzen. In Experimentaluntersuchungen liess sich zeigen, dass die beim Einstich durch Pflanzenläuse hervorgerufenen physiologischen Störungen des Wirtspflanzengewebes ebenso durch die Zugabe definierter Lösungen der analysierten freien AS des Speichels (einzeln und bes. im Gemisch) ausgelöst werden können.

Im einzelnen erzeugen sie folgende Reaktionen: Erhöhung der Plasmaströmung der pflanzlichen Zelle. Weiterhin werden Wasseraufnahme und Transpiration der pflanzlichen Organe durch die analysierten AS gestört, auch liess sich eine Beeinflussung der Respiration (Erhöhung) und der Photosynthese (Hemmung) zeigen.

Inzwischen fand Anders (1957-59) bei der Reblaus, dass die freien AS des Speichels dieser Art für deren cecidogene Wirkung verantwortlich sein sollen, was nicht verwunderlich ist, nachdem AS in sehr geringen Konzentra-

tionen Wachstumswirksamkeit bei Keimlingen höherer Pflanzen (Reinholz, 1954) zeigen. Schäller (1960) fand bei der gleichen Art jetzt 7 freie AS bzw. Amide. Die Phytopathogenität von Aphiden scheint mit dem AS-Gehalt ihres Speichels geradezu korreliert zu sein, die erwähnte Fichtenlaus *Elatobium abietinum*, bei der wir 7 freie AS fassen konnten, ruft besonders starke Schadeffekte hervor (Kloft, Ehrhardt, 1959), ähnliches gilt nach noch unpubl. Arbeiten meines Schülers P. Ehrhardt auch für Lachniden. Hier konnten bei den Wucherungen hervorrufenden Arten der Gattung *Schizodryobius* 6 freie AS festgestellt werden, während sich bei 2 als völlig indifferent geltenden Koniferen-Lachniden-Arten bisher keine oder nur ganz geringe Mengen freier AS finden liessen.

Es dürfte lohnend sein, die Bedeutung von mit dem Speichel oder anderen Sekreten ausgeschiedenen freien AS auch bei weiteren phytopathogenen Insekten zu überprüfen.

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ZUSAMMENFASSUNG

Mit Hilfe papierchromatographischer Analysenmethoden wurden die freien Aminosäuren im Speichel plantisuger Insekten semiquantitativ analysiert. Während sich der Speichel von Heteropteren direkt durch Punktierung des Reservoirs der Hauptspeicheldrüse gewinnen liess, wurde der Speichel von Aphididen durch Anstich der Läuse direkt auf den Startpunkt des Chromatographiepapiers (bei Grünlicht auslösbar) gewonnen, bei Parenchyms augenden Cocciden konnte Speichel z. T. direkt an der Rüsselspitze abgenommen werden. Im einzelnen konnte ein Spektrum bis zu 13 freien Aminosäuren bei Wanzen, bis zu 8 bei Aphiden im Speichel nachgewiesen werden. Die Phytopathogenität von Aphiden und der Gehalt ihres Speichels an freien Aminosäuren scheinen in Relation zu stehen, da die freien Aminosäuren phytotoxische Wirkungen besitzen. Hierher gehören nach eigenen Untersuchungen vor allem stoffwechselphysiologische Störungen und Wachstumseffekte, nach anderen Arbeiten auch cecidogene Wirkungen.

RIASSUNTO

Aminoacidi liberi come sostanze fitopatologiche attive nella saliva di Insetti succhiatori di linfa.

Con l'ausilio di metodi di analisi cromatografica su carta si analizzarono gli aminoacidi liberi nella saliva di Insetti succhiatori di linfa in modo semiquantitativo.

Mentre la saliva di Eterotteri potè essere ottenuta direttamente per puntura del serbatoio della glandola salivare principale, quella degli Afidi fu ricavata per puntura degli Insetti stessi direttamente sulla carta per cromatografia (ottenuta con luce verde) e quella dei Coccidi succhiatori di parenchima in parte direttamente all'estremità del rostro.

In particolare si potè ottenere uno spettro comprendente fino a 13 aminoacidi liberi nelle Cimici e fino a 8 negli Afidi. La fitopatogenicità degli Afidi e il contenuto in aminoacidi liberi della loro saliva sembrano essere in relazione, poichè gli aminoacidi liberi hanno azione fitotossica. Secondo ricerche personali, questa azione si manifesta soprattutto con disturbi nella fisiologia del metabolismo e con effetti sulla crescita, secondo altri lavori anche con manifestazioni cecidogene.

LEVINSON Z. H. (*)

THE FUNCTION OF DIETARY STEROLS IN PHYTOPHAGOUS INSECTS (**)

Bergmann and Levinson (1958) have shown that the sterols extracted from *Musca vicina* Macq., *Colias hyale* Godt. and *Orgyia antiqua* L. which had been reared on vegetarian diets, are nutritionally equivalent to cholesterol or 7-dehydrocholesterol for larvae of *Dermestes vulpinus* Fabr. This confirms the finding of Beck and Kapadia (1957) who detected these sterols in *Tribolium confusum* Duval by chemical methods. More recently Clark and K. Bloch (1959) succeeded in isolating and identifying 22-dehydrocholesterol from *Blattella germanica* which had been grown on a synthetic diet containing uniformly labeled ergosterol.

In the present investigation, sterol conversion was examined in a range of insects which had been reared on their normal plant food or on artificial diets. Fresh plant material and larvae of the final instar were used throughout. The lipids of the dessicated, powdered plants or diets and insects were obtained by exhaustive extraction with hot acetone, chloroform and ether and were saponified by boiling ethanolic KOH; the sterols were precipitated from the resulting unsaponifiable fractions as digitonides and subsequently recovered in the free form (Schoenheimer and Dam 1933).

Sterols and unsaponifiable fractions (USF) were assayed biologically using larvae of *D. vulpinus*. The newly hatched larvae were placed on a semisynthetic diet (Fraenkel 1959) in which cholesterol was replaced by the sterol or USF under test. The stock culture medium served as the control diet; it consisted of 75 pts. anhydrous fishmeal, 17 pts. liver powder, 3 pts. dried yeast and 5 pts. water. The beetles were given an additional amount of water to provoke oviposition.

Newly hatched larvae of *D. vulpinus* died on the semisynthetic diet deficient in cholesterol or 7-dehydrocholesterol after 2-3 molts. In the presence of

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0.32 % dietary cholesterol the larvae grew as well as on the control diet and pupated within an average of 37 days. No pupation occured in the presence of plant sterols listed in Table 1. These results are in agreement with our previous findings where a different diet was employed.

TABLE 1.

Utilization of some purified sterols by Hide Beetle larvae.

Sterol added (0.32 % to lipid free diet)	No. larvae reared	No. larvae pupating	Avg. time (days)	
			to pupation	to death
β-Sistosterol	20	0	—	39
γ-Sistosterol (= Clionasterol)	20	0	—	33
Ergosterol	15	0	—	24
Stigmasterol	25	0	—	28
Brassicasterol	10	0	—	
Cholesterol	25	25	37	—
none	20	0	—	21

T = 29 ± 0.5°, RH = 65 ± 5 % in all experiments

The plant sterols appeared, however, not to be entirely inert as in their presence the dermestid larvae survived longer than on a sterol-free diet. Whether this effect is due to an extremely slow conversion or to a synergistic relationship with endogenous cholesterol (from the egg stage) remains to be investigated.

Fig. 1 summarizes the results of an attempt to determine the threshold concentration of dietary cholesterol required by hide beetle larvae. It was found that when 12 g. diet containing only 0.008 % cholesterol were offered to twenty larvae, a small percentage of them still pupated, although with considerable delay. These results are in general quantitative agreement with the findings of Clark and Bloch (1959). There is an obvious tendency for cannibalism among larvae that were grown on low levels of cholesterol. This behaviour may not be symptomatic of cholesterol deficiency only, but rather a response of gregarious insects to partial lack of nutrients in general. Nevertheless, a cannibalistic larva acquires from the bodies of its litter mates the greatest possible concentration of the indispensable nutrient(s).

The effect on *D. vulpinus* of sterols or unsaponifiable fractions derived from various insect species and their respective foodstuffs was compared and the experimental results are given in Table 2. While none of the plant-derived sterols supported pupation of *Dermestes* larvae, all the insect preparations did. The variations between pupation times suggest low and high conversion rates for the phytosterol ingested by different species.

It was subsequently found more convenient to assay sterols from various sources without going through the extraction procedure. Dessicated, powdered insects supplemented with B-vitamin solution (10 %) were used directly as test diets for *Dermestes* larvae. The results recorded in Table 2 were confirmed by this simplified method and also further extended to include diets prepared from other vegetarian species, including a crustacean and a mollusc (Table 3).

Furthermore, cholesteryl chloride, a specific cholesterol antagonist for housefly larvae (Levinson and Bergmann 1957), proved to interfere also with the utilization of the tissue sterol of *Tenebrio molitor* by dermestid larvae. Moreover, the inhibitory effect could be completely reversed by an excess of Tenebrio USF (Table 4).

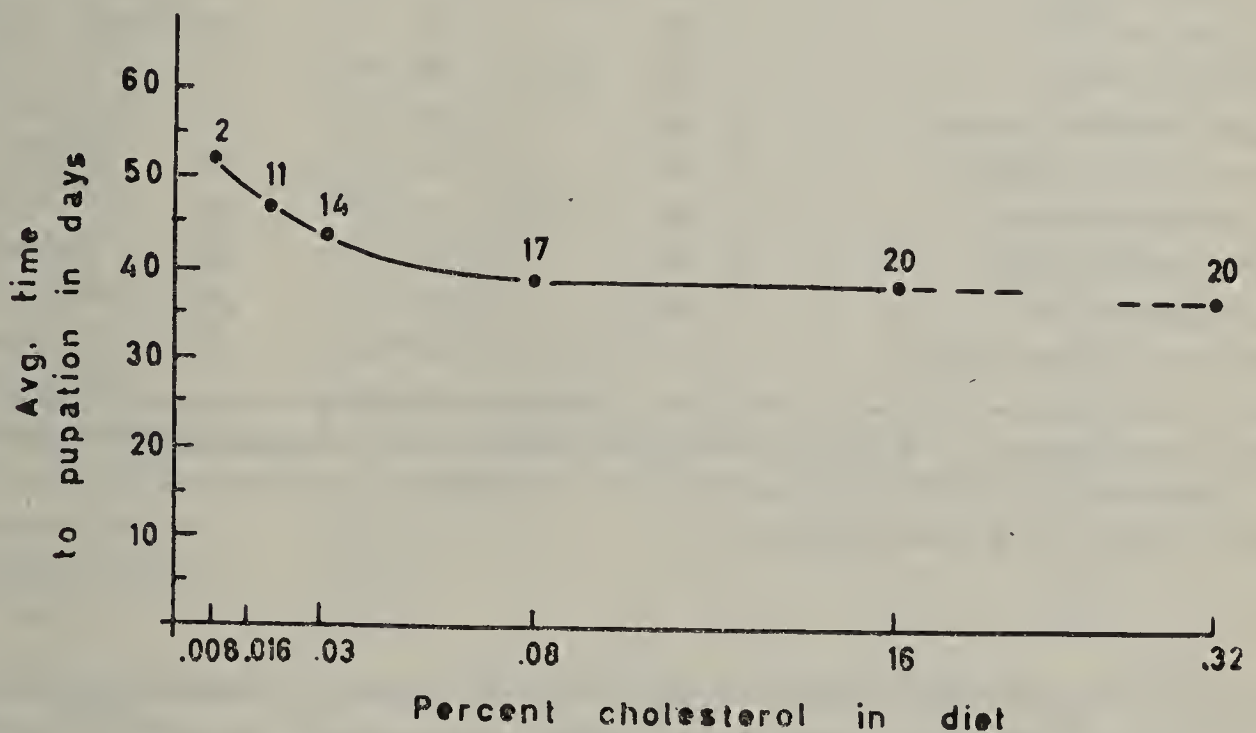

Fig. 1.
Pupation of *D. vulpinus* larvae in relation to dietary cholesterol.
The numbers on the curve represent the numbers of pupae formed from 20 larvae.

The sterols of several insects species and their diets were subjected to chromatographic separation on paper using a modification of the method of Kodicek and Ashby (1954). Amounts of the order of 10 μg of cholesterol and 7-dehydrocholesterol could be readily separated from either β-sitosterol or stigmasterol and somewhat less completely from brassicasterol. The relative position of the sterol fronts (cholesterol = 1.0) was 1.12 for 7-dehydrocholesterol, 0.87 for β-sitosterol, 0.90 for stigmasterol and 0.91 for brassicasterol. The food sterols gave single spots located in the position of β-sitosterol, whereas the insects sterols revealed, except for *Schistocerca*, two spots, one of which ap-

TABLE 2.

Effect of sterols derived from some insects and their food plants on *D. vulpinus* larvae.

Addition to diet (1)	No. larvae reared	No. larvae pupating	Average time (days) to pupation	to death
Wheat bran sterols	20	0	—	43
Ligustrum vulgare sterols	10	0	—	45
Brassica oleracea sterols	15	0	—	39
Whole meal flour USF	20	0	—	46
Brassica campestris USF	15	1 (2)	—	45
Urtica urens USF	10	0	—	38
Gossypium sp. USF	25	0	—	36
Wheat shoots USF	10	0	—	41
Dixippus morosus sterols	10	9	45	—
Philosamia ricini sterols	15	14	42	—
Pieris brassicae sterols	10	9	46	—
Tenebrio molitor USF	15	15	38	—
Earias insulana USF	20	18	48	—
Schistocerca gregaria USF	10	10	37	—

(1) Dietary concentrations = 0.32 % for sterols or 2.00 % for unsaponifiable fractions (USF) (USF contained 20.0 - 33.0 % 'phytosterols' or 10.5 - 15.0 % 'zoosterols')

(2) abnormal pupa; died subsequently.

peared to be the ingested plant sterol and one closely resembling cholesterol (Fig. 2). The food sterol was converted either moderately, largely or completely to cholesterol.

It appears, therefore, that conversion of plant sterols to cholesterol occurs in a broad variety of species belonging to the orders of *Coleoptera, Diptera, Hymenoptera, Lepidoptera* and *Orthoptera*. The conversion of the principal phytosterols to cholesterol would involve the following chemical changes: (1) Elimination of the methyl group of campesterol or the ethyl group of either β-sitosterol or clionasterol (γ-sitosterol) from position 24 of the side chain. (2) Saturation of the $\Delta^{22:23}$ double bond together with elimination of the C_{24} methyl group of brassicasterol or the C_{24} ethyl group of stigmasterol. (3) Saturation and deethylation of the side chain together with altering the position of the double bond in ring B of α-spinasterol. There is an urgent need for work which would lead to an understanding of the underlying mechanism of these changes.

The dependence of sterol utilization on the feeding habits of insects has been demonstrated (Levinson 1955). This dependence can now be interpreted

Table 3.

Growth of *Dermestes vulpinus* on powdered insects.
Groups of 5 larvae were reared on 2.7 g anhydrous larval powder supplemented with 0.3 ml B-vitamin solution.

Organism investigated	respective food	No. larvae reared	No. larvae pupating	Average time (days) to pupation	Average time (days) to adult emergence
Phaedon cochleariae	Brassica campestris	15	14	25	31
Tenebrio molitor	Wheat bran	30	30	19	24
Tribolium confusum	Wholemeal flour	25	25	20	25
Calliphora erythrocephala	Synthetic diet (1)	35	33	23	28
Musca domestica	Synthetic diet (2)	25	24	22	28
Apis mellifera	Royal yelly,	25	17	29	35
Bombus sp.	Pollen + Honey	10	8	28	34
Aglais urticae	Urtica urens	20	19	22	27
Earias insulana	Gossypium sp.	25	25	21	26
Philosamia ricini	Ligustrum vulgare	15	15	24	29
Pieris brassicae	Brassica oleracea	25	23	23	28
Dixippus morosus	Ligustrum vulgare	30	29	23	29
Locusta migratoria	Wheat shoots	25	25	20	25
Schistocerca gregaria	Wheat shoots	35	35	19	25
Armadillium vulgare	Molds	10	8	31	37
Helix aspersa	Leaves	10	10	29	35

(1) Sedee (1956).

(2) Levinson (1958); the larvae of both species were grown under aseptic conditions with β-sitosterol as the only dietary sterol.

in terms of the inability or ability of the diverse species to convert different sterols to cholesterol. The latter is most likely to be a general attribute of phytophagous species, which was lost after the carnivorous habit had been acquired. It is interesting to note that species which are both phytophagous and zoophagous, like *Musca* and *Calliphora*, convert β-sitosterol less effectively than the obligatory plant feeders.

In view of the present findings, it seems to be desirable to recapitulate the functional aspects of cholesterol in insects. Apart from being a growth factor for larvae, this compound is also required for normal reproduction (Chauvin 1949, Monroe 1959). Partial deficiency in cholesterol or its dietary precursors not only curtails the growth of larvae, but also deprives them of their natural

immunity towards bacterial infection, as illustrated by work on *Lucilia sericata* (Hobson 1935) and *Musca vicina* (Silverman and Levinson 1954). The facts, that the need for cholesterol decreased with increasing larval age of *Tenebrio molitor* (Leclerq 1948) and *Calliphora erythrocephala* (Levinson, unpublished observation) and that the sterol content of *Tenebrio molitor* (Finkel 1948) and *Musca vicina* (Levinson and Silverman 1954) remained unchanged during metamorphosis, suggest effective storage by larvae and pupae.

TABLE 4.

The Effect of Cholesteryl Chloride on Tenebrio-Sterol Utilization by *Dermestes vulpinus.*

Cholesteryl chloride mg/diet/larva	Average weight in mg				% weight suppression	Average time to pupation in days
	age in days			of beetles at emergence		
	10	16	22			
0.0	16.5	39.8	41.0	29.0	0	23
3.1	14.3	33.5	34.3	19.5	33	25
6.2	5.3	26.0	28.8	15.6	56	28
9.0	1.5	larval mortality after 12 days				
12.5	0.5					
6.2 + 30 mg Tenebrio-USF	17.2	40.1	39.5	30.5	0	23

Several cholesterol homologs having less than eight carbon atoms in their side chain appeared to act as sparing agents for cholesterol in housefly larvae (Bergmann, Rabinovitz and Levinson 1958), as did cholestan-3β-ol, Δ^7-cholestenol, 22-dehydro-cholesterol and Δ^7-ergosterol for *Dermestes* larvae (Clark and Bloch 1959). It has been suggested for this reason that cholesterol may have a metabolic role, whilst the closely related « sparing » sterols are used for structural purposes.

There is evidence that cholesterol may be a source of steroid hormones. An extract prepared from ovaries and oviducts of freshly emerged females of *Attacus atlas* was found to excert estrogenic activity in mice and the concentration of the estrogen in the moth ovaries closely resembled that in mice ovaries, i. e. 90-130 mouse units/kg of fresh tissues (Loewe, Raudenbusch and Voss 1932).

The juvenile hormone (neotenin) was first recognized by Wigglesworth (1934, 1936). This agent was found to promote larval development but to prevent metamorphosis and to be required for yolk deposition in the egg. It is

remarkable that this type of activity closely parallels the dietary function of cholesterol. Moreover, extracts of the adrenals of cattle (Gilbert and Schneiderman 1958) as well as the unsaponfiable fat of *Hyalophora cecropia* (Scheiderman and Gilbert 1957) were found to contain substances with juvenile hor-

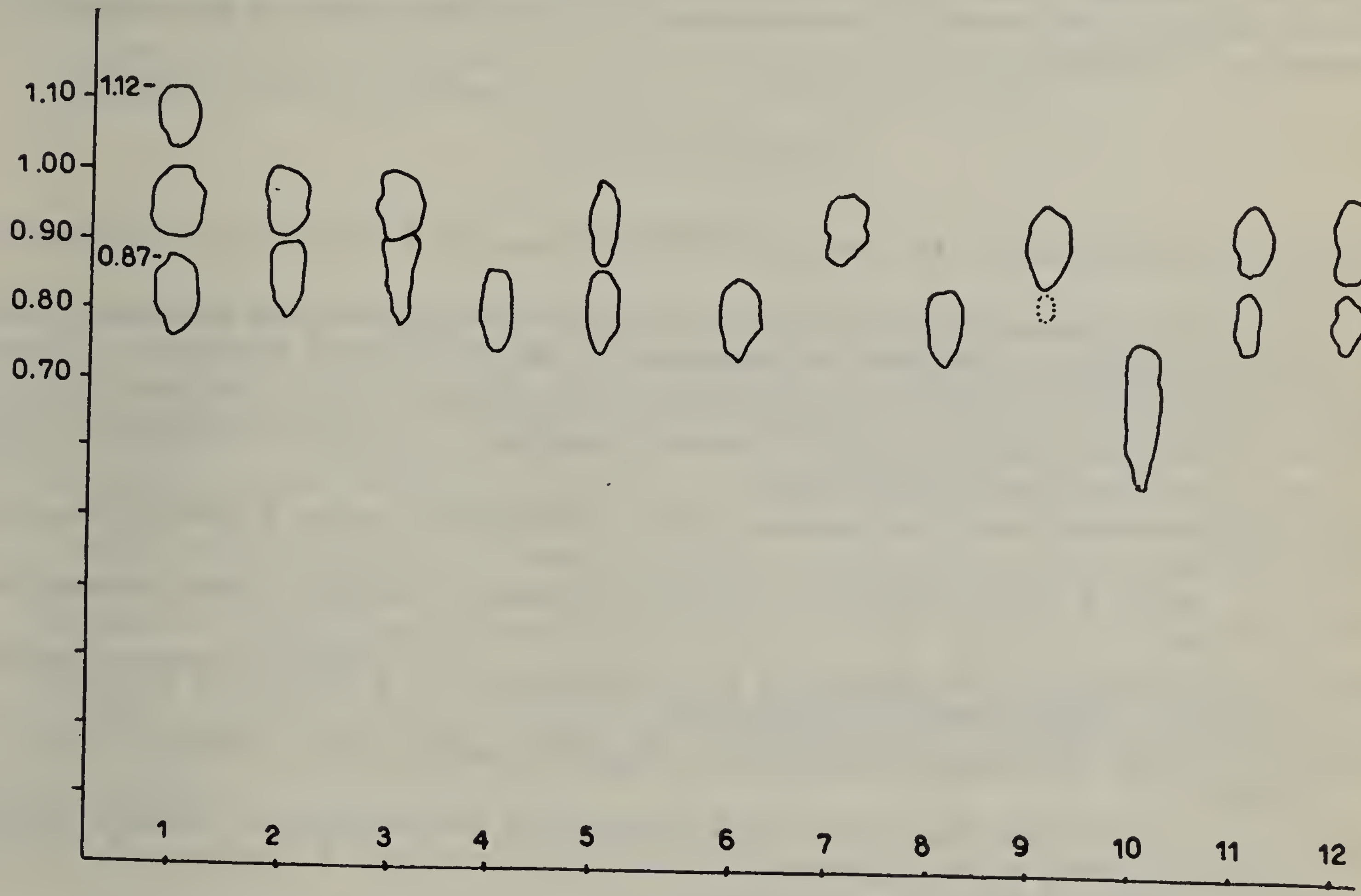

Fig. 2.

Diagram of chromatograms of sterols from several insects and their food plants.

1. R Cholesterol = 1.00
   7-Dehydro-cholesterol = 1.12
   β-Sistosterol = 0.87
2. Cholesterol, Stigmasterol
3. Cholesterol, Brassicasterol
4. *Urtica*-sterol
5. *Blissus*-sterol
6. Wheat-sterol
7. *Schistocerca* sterol
8. *Brassica oleracea* sterol
9. *Pieris* sterol
10. *Ligustrum* USF
11. *Philosamia* sterol
12. *Dixippus* sterol

mone activity. Preliminary experiments with unsaponifiable fractions from the larval lipids of *Blissus, Pieris* and *Schistocerca* carried out recently by H. A. Schneiderman reveal neotenin activity when tested on pupae of *Antheraea polyphemus.*

In spite of this knowledge, the physiological function of cholesterol cannot yet be precisely defined.

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SUMMARY

The tissue sterols of phytophagous larvae were compared with those of their food by means of 1) a bioassay based on the inability of *Dermestes vulpinus* to reach the pupal stage in presence of dietary C_{28} and C_{29} sterols and their strict requirement for either cholesterol or 7-dehydrocholesterol, and 2) chromatographic separation on paraffin-impregnated filter paper.

Phytophagous species distributed among *Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera* and *Orthoptera*, a Mollusc and a Decapod convert at varying extent the C_{28-29} sterols of their food to cholesterol, whereas obligatory carnivora like *Dermestes* or *Attagenus* are incapable of doing this.

Utilization of *Tenebrio*-body sterol (derived from dietary sitosterol) is reversibly inhibited by cholesteryl chloride.

Cholesterol is required for larval growth and for oogenesis; its function resembles that of the juvenile hormone, which is recognized as an unsaponifiable constituent of insect lipids. Cholesterol may act as a precursor of steroid hormones, in addition to its structural and anti-infective role.

RIASSUNTO

La funzione degli steroli della dieta negli Insetti fitofagi.

Gli steroli tessutali della larva fitofaga sono comparati con quelli del loro cibo per mezzo di: 1) Saggio biologico basato sulla incapacità di *Dermestes vulpinus* di giungere allo stadio pupale in presenza di steroli C_{28} e C_{29} nella dieta ed esatta richiesta di colesteroli o di 7-deidro-colesterolo, e 2) separazione cromatografica su carta da filtro impregnata di paraffina.

Le specie fitofaghe appartenenti a *Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera* e *Orthoptera*, a Molluschi e Decapodi, trasformano in vario grado lo sterolo C_{28-29} del loro cibo in colesterolo, mentre i carnivori come *Dermestes* o *Attagenus* sono incapaci di fare ciò.

L'utilizzazione degli steroli del corpo di *Tenebrio* (derivati dal sitosterolo della dieta) è reversibilmente inibita dal cloruro di colesterile.

Colesterolo è richiesto per lo sviluppo larvale e per l'oogenesi; la sua funzione assomiglia a quella dell'ormone giovanile che è dimostrato essere un costituente insaponificabile dei lipidi degli Insetti. Il colesterolo può essere considerato come precursore di ormoni sterolici in aggiunta al suo significato strutturale e antinfettivo.

Levinson Z. H. (*)

THE EVOLUTION OF STEROL REQUIREMENTS (**)

The nutritional requirements of the organism studied so far, reveal peculiar phyletic differences for the sterols.

The majority of bacteria neither need nor synthesize sterols for their growth and reproduction (1), they lack the oxidocyclases essential for sterol formation (2). Yet the acetate requirement for growth of certain *Lactobacilli* is replaceable by mevalonic acid (3). One may assume that lipids other than sterols could fulfil the function of the latter in *Bacteria.* However, fungi, lichens and algae differ distinctly from bacteria by their ability to synthesize ergosterol, zymosterol and some of their homologs (4). The primitive asexual *Myxophyceae*, on the other hand, do not produce any sterols (5). Higher plants synthesize mainly C_{28-29} Δ^5 - and Δ^7 - sterols (4).

Several genera of *Protozoa* (6) have specific sterol requirements, which can be met by cholesterol, except for *Paramecium* aurelia that utilizes C_{29}-sterols only (7).

Among the *Metazoa*, the annelid *Lumbricus terrestris* is capable of converting mevalonic acid to squalene only; sterol biogenesis is interrupted at this stage (8). In the insects, the phylogenetic descendants of *Annelidae*, sterol synthesis is blocked at a considerably lower level: Larvae of *Dermestes vulpinus* cannot incorporate acetate or fructose to squalene (9). The indispensability of cholesterol for a mollusc, *Helix pomatia* has also been established (10).

It remains to be clarified whether the diverse sterols found in the tissues of *Porifera, Coelenterata* and *Echinodermata* (11) are in fact synthesized by these forms or derived from exogenous sources.

The sterol requirement of insects may manifest itself at different developmental stages, perhaps due to varying storage of cholesterol. Indispensability of the sterol becomes evident in the early postembryonal growth of *Calliphora* and *Tenebrio* larvae (12), prior to puparium formation of *Dermestes* (13), and in the course of oogenesis in *Blattella, Dermestes* and *Musca* (14).

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With regard to their sterol utilization, the larvae of insects may be divided into two major groups:

a) Phytophaga capable of converting the C_{28-29} sterols of their food plants to cholesterol.

b) Carnivora adapted to cholesterol utilization only (13).

It is interesting to note that *Blattella* and *Calliphora* shorten the side chains of dietary ergosterol and β-sitosterol by demethylation (15) or deethylation (16), respectively.

The pathway of cholesterol synthesis by vertebrates has been amply demonstrated in mammals and birds (17). Although these animals have evolved enzyme systems that enable them to produce cholesterol from two-carbon units, they depend on sterols of the vitamin D group or their precursors (18). Guinea pigs need in addition either stigmasterol or β-sitosterol (19), the dominant sterol constituents of their food.

The function of cholesterol in cuticle sclerotization of certain insects (20) has perhaps an analogy in the skeleton calcification of vertebrates promoted by chole- or ergocalciferol.

Thus it would seem that the evolution of sterol requirements is still evident in the highest animal phyla.

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SUMMARY

The nutritional requirements of the organism studied so far, reveal peculiar phyletic differences for the sterols. These differences are summarized.

RIASSUNTO

Evoluzione del bisogno in steroli nel regno animale.

Il bisogno nutritivo di steroli negli organismi animali finora studiati, rivela caratteristiche particolari che sono in relazione con la sistematica zoologica. Tali caratteristiche vengono indicate riassuntivamente.

Ito T. (*)

AN ARTIFICIAL DIET FOR THE SILKWORM, *BOMBYX MORI*, AND THE EFFECT OF SOYBEAN OIL ON ITS GROWTH

Information on nutritional requirements and artificial diets is available for several species of phytophagous insects, but most of them are not typical leaf feeders. The silkworm has, so to speak, a distinctive food habit and has been highly domesticated over hundreds years and consequently bred so as to produce large amount of silk substances. All of the trials to apply diet formulae reported for other insects have been unsuccessful for the silkworm.

Recently, it has been reported that silkworm larvae can be reared entirely on artificial diets, when they contain a high amount of mulberry leaf powder, with other suitable ingredients, such as, sucrose and soybean powder (Fukuda *et al.*, 1960; Ito and Tanaka, 1960; Yoshida *et al.*, 1960). The present author subsequently tried to improve on previous diet in two respects; decreasing the amount of leaf powder to the level at which the larva still shows satisfactory feeding reaction, and replacing impure substance, soybean powder, by possibly pure compounds. Up to the present time, it is still unsuccessful to prepare the diet for the silkworm, which does not contain leaf powder at all. In the course of diet improvement, however, it was noticed that the diet containing 10 % leaf powder practically did not disturb the results of nutrition experiments. The use of defatted soybean powder was subsequently shown to inhibit larval growth and development, which however were resumed by the addition of soybean oil (Ito, 1960 a). Further improvement of the diet is being carried out in my laboratory, and the recent results, especially the role of soybean oil as one of growth promoting substances, will be worthwhile to be reported here.

PREPARATION OF ARTIFICIAL DIET

The diet used in those studies is a culturing medium solidified with potato starch. Its composition is presented in Table 1. This diet is slightly modified from that used in an earlier work (Ito, 1960 a); the amount of cellulose powder is raised this time from 14 to 30 %, since the feeding has been shown to be markedly accelerated by increasing its concentration (Ito *et al.*, 1960; also cf.

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Ito, 1960 b). The vitamin mixture used is shown in Table 2. The method used in preparing the diets and maintaining the cultures has been described in detail elsewhere (Ito, 1960 a, 1960 c). The criteria used for the evaluation of nutritive value were the survival, weight increase, and instar progression.

REQUIREMENT FOR SOYBEAN OIL AND THE EFFECT OF ITS CONCENTRATION

The nutritive effect of soybean oil was at first shown to decrease with refining processes, therefore, the degummed oil, not refined, was used for nutri-

TABLE 1
Composition of basal diet used to test nutritive effect of soybean oil.

Substance	Amount used g	Dry diet %
Mulberry leaf powder	0.6	10
Starch (potato)	0.9	15
Sucrose	1.32	22
Soybean casein (defatted)	1.32	22
Cellulose powder (*)	1.8	30
Wesson's salt mixture (*)	0.06	1
	6.0	100
Vitamin mixture	2.37 mg	
Distilled water (total)	9.0 ml	

(*) Product of General Biochemicals, Inc., Ohio, U.S.A.
When adding soybean oil or phosphatides, the corresponding amount of sucrose plus casein (1:1) was subtracted to maintain the correct volume.

TABLE 2
Composition of the vitamin mixture.

Vitamin	Amount added to 6 g dry diet µg
Biotin	10
Calcium pantothenate	100
Choline chloride	1,000
Folic acid	10
Inositol	1,000
Niacin	100
Pyridoxine HCl	50
Riboflavin	50
Thiamine HCl	50
TOTAL	2,370

Added to the diet in an aqueous solution.

tional test of the oil. As shown in Table 3, when the diet did not contain any soybean oil, the larva neither survived after 15-day rearing nor reached the third instar within this period. Adding the oil to the diet, there appeared some larvae which passed through three larval instars and reached the fourth instar in 15 days, with a concomitant of good survival. The best larval survival

TABLE 3

Effect of concentration of soybean oil on growth, development, and survival of the silkworm. Experiment was run in a duplicate, each 20 larvae (newly hatched).

Concentration of oil (*) %	No. of surviving larvae after 15 days	Average weight mg	No. of larvae reached through various instars in 15 days	
			3rd instar	4th instar
0	0 (**)	—	0 (**)	— (**)
4	23	14.9	24	4
8	36	46.1	35	7
12	29	38.7	31	6
16	3	46.5	5	2

(*) Degummed soybean oil.
(**) Larval number out of 40 (sum of duplicate).

and weight increase were obtained on the 8 % diet. The 12 % diet was inferior to this. Only three out of forty larvae survived on the 16 % diet. Thus, the nutritive effect of the oil increases with increasing ratio of it in the diet, but there is an optimal concentration for the best larval growth, and further addition beyond this level is rather inhibitory.

EFFECT OF SOYBEAN STEROLS AND SOYBEAN PHOSPHATIDES

Some kinds of sterols have reducing or inhibiting effects on feeding of the silkworm, which however can be minimized by the accompaniment of soybean oil (Ito, 1960 d). It is, therefore, necessary to discriminate nutritive effect from feeding reaction, especially in the experiment on sterols. Soybean sterols (m. p., 136 - 7° C) have little inhibitory effect on feeding (Ito, 1960 d), but the nutritional test with them was carried out in combination of the presence of soybean oil. The oil used for this purpose was one of the most refined ones obtained commercially, 99.5 % of it being fatty acid glycerides. The nutritive effect of this oil for the silkworm was rather poor, as compared to degummed

oil, unrefined one (Table 4). The addition of 6 mg soybean sterols to 6 g dry diet was very much effective for larval growth and development, as is evident from Table 4.

TABLE 4

Effect of soybean sterols and of soybean phosphatides on growth, development, and survival of the silkworm. Experiment was run in a duplicate, each 20 larvae (newly hatched).

Substances contained in diet	No. of surviving larvae after 15 days	Average weight mg	No. of larvae reached through various instars in 15 days	
			3rd instar	4th instar
No addition	1 (*)	1.8	0 (*)	— (*)
2 % oil (**) (data after 17-day rearing)	1	4.4	1	0
2 % oil (**) + 6 mg soybean sterols	33	30.7	38	3
No addition	0	—	0	—
1 % soybean phosphatides . .	34	29.0	32	3
No addition	0	—	0	—
4 % soybean fatty acids (***) .	1	3.6	0	—
4 % soybean fatty acids (***) + 20 mg unsaponifiable matter	23	7.4	18	0

(*) Larval number out of 40 (sum of duplicate).
(**) The most refined soybean oil, obtained commercially, was used.
(***) Obtained after saponification.
Soybean sterols and unsaponifiable matter were added to 6 g dry diet, respectively.

Soybean phosphatides (P, 3.2 %; N, 1.1 %) were then tested for their nutritive effect. From the data shown in Table 4, it is concluded that soybean phosphatides are also of high nutritive value at 1 % level (60 mg added). There has been so far no report distinctly demonstrating the importance of phosphatides in the nutrition of phytophagous insects.

Table 4 further shows that soybean fatty acid mixture, obtained after saponification, has little nutritive effect for the silkworm. The nutritive effects of soybean oil are thus rather concentrated to the unsaponifiable matter, as shown in Table 4. This is exactly in accord with the findings that the effect of soybean sterols is very high, and that the effect of soybean oil decreases according to the refining processes (Ito, 1960 c).

STEROL REQUIREMENT OF THE SILKWORM

It has been established that insects require dietary sterols for normal growth, and there is so far no exception to this point. An artificial diet of such a composition as shown in Table 1 was also used for testing the effect of sterols for the silkworm. The results showed that silkworm larva required cholesterol, ergosterol, β-sitosterol, and stigmasterol. The best growth and survival were obtained with either of latter two substances (Ito, 1960 d).

The details of sterol requirements as well as nutritive effect of soybean oil in relation to diet improvement will be reported elsewhere.

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SUMMARY

A diet was described on which the larvae of the silkworm show suboptimal growth. This diet consists of mulberry leaf powder, starch, sucrose, soybean casein, soybean oil, soybean sterols, Wesson's salt mixture, vitamins, and cellulose powder. Without soybean oil and sterols, only poor growth and development and low survival were obtained. Soybean oil supplied necessary growth substances. The effect of the oil was concentrated in unsaponifiable matter, not in fatty acid mixture. The nutritive effect of both soybean sterols and phosphatides were remarkable. Finally, the requirement for various sterols was determined.

RIASSUNTO

Una dieta artificiale per il baco da seta Bombyx mori *e l'effetto dell'olio di soia sulla crescita.*

Si descrive una dieta con cui le larve del baco da seta hanno una crescita subottimale. Questa dieta consiste in polvere di foglie di gelso, amido, saccarosio, caseina di soia, olio di soia, steroli di soia, una miscela di sale di Wesson, vitamine, e polvere di cellulosa. Senza l'olio di soia e gli steroli, si ottennero solo debole crescita e sviluppo con una bassa sopravvivenza. L'olio di soia fornì sostanze necessarie alla crescita. La parte attiva dell'olio fu concentrata nella frazione insaponificabile, non nella miscela di acidi grassi. L'effetto nutritivo degli steroli di soia e dei fosfatidi fu notevole. Ed in ultimo si stabilì la necessità di vari steroli.

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SILKWORM RAISING ON ARTIFICIAL FOOD

II. THE IMPROVEMENT OF THE FOOD COMPOSITION

Yoshida, Matsuoka and Kimura (1960) have succeeded in rearing silkworms on artificial food, from hatching to the fourth day of the fifth instar (maximum), but they could not get the silkworms to produce cocoons. The authors (1960) reared silkworms by improving the composition of the artificial food and the method of the silkworm raising devised by Yoshida *et al.*, and succeeded in making silkworm larva produce 36 cocoons, starting from 110 worms hatched from eggs. The silkworms turned to pupae, and the fertilized moth laid their eggs. Ito and Tanaka (1960) also succeeded, about two months after our success, in rearing silkworms on an artificial food whose composition differs a little from the author's one. However not only our experiment but also that of Ito and Tanaka showed that results of silkworm raising obtained with these artificial foods are remarkably inferior to ones obtained with fresh mulberry leaves. In the current paper we report on the composition of the improved artificial food and on results of silkworm raising obtained with it.

PREPARATION OF ARTIFICIAL FOOD AND SILKWORM RAISING

Preparation of artificial food: To a beaker containing 5.5 g of powdered mulberry leaves, 1.5 g of potatoe starch and 1 g of « Kinako » (panched soy bean) were added 5 ml of 20 per cent sucrose solution and 10 ml of the inhibitor solution. The mixture was stirred well with a glass rod. The artificial food prepared was sterilized in an autoclave for 15 minutes, and stored in a refrigerator. The artificial food freshly prepared is good for a week. Powdered mulberry leaves was prepared by powdering mulberry leaves dried at not over

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40° C under vacuum to pass through an 80 mesh sieve. The mulberry leaves were picked from mulberry trees « Kokuso » No. 19 in May 1960. The potatoe starch and « Kinako » were respectively commercial, and they were powdered to pass through an 80 mesh sieve. The inhibitor solution which is necessary to stabilize the artificial diet, and to prevent its decomposition was prepared by dissolving 0.1 g of vitamin K_3, 0.2 g of sodium dehydroacetate and 0.2 g of sodium sorbate in 100 ml of distilled water by heat.

Silkworm raising: A piece of wet filter paper was placed in the bottom of a petri dish. This was covered with a piece of paraffin paper. The artificial food was placed on the paraffin paper, then the newly hatched worms, the hybrides between Shi 115 X Shi 124 and Nichi 122 X Nichi 124, were placed in a box at a temperature 23° C and a humidity 92-96 per cent. The petri dish in which silkworms from hatching to the end of the third instar are reared was always put on the lid. The artificial was cut in 0.1 mm $\sim$ 0.3 mm thick. The feeding of the slices of the artificial food to the silkworms was done twice daily at 12 hour intervals for silkworms from hatching to the end of the third instar; 3 times daily at 8 hour intervals for silkworms from the beginning of the fourth instar to the end of the fifth instar.

RESULTS AND DISCUSSION

The elapsed time, silkworm larva hatching to spinning a cocoon, was about 50 days. This value is about twice, that of the same race reared on fresh mulberry leaves. The larval and pupal period of the silkworm reared on the present artificial food is also almost the same as compared with that of silkworm reared on the first artificial food adopted in a previous paper. The composition of the artificial food adopted in the previous paper consists of 5 g of powdered mulberry leaves, 1.5 g of potatoe starch, 1 g of « Kinako », 1 g of « Koridofu » (non-fat frozen soy bean curds), 5 ml of 20 per cent sucrose solution and 10 ml of the inhibitor solution. With the present artificial food and the present silkworm raising method, we could not shorten the larval and pupal period, especially larval one, of the silkworm. Recently the authors (unpublished) reared the same race of silkworms on the same diet, but by the different silkworm raising method (the feeding of the diet to the silkworms was made 6 times daily at 4 hour intervals). As a result, the larval period of the silkworm was shortened and became 30 days. Among 100 new hatched silkworms, 42 worms spun their cocoons, and 42 worms underwent the pupal stage (♀ 15 and ♂ 22). In one case the artificial food adopted in the previous paper was used, 10 worms spun their cocoons starting from 100 worms hatched newly from eggs, and 10 worms underwent the pupal stage (♀ 1 and ♂ 9) and

10 worms to the moth (♀ 1 and ♂ 9). The weight of the cocoon fibres produced by one silkworm was 112 mg (148 mg maximum), and its value is about 1.4 times as compared with that of the silkworm reared on the artificial food adopted in the previous paper, being about 25 per cent (35 per cent maximum) of that in the case of the same race reared on fresh mulberry leaves. The length of a silk filament unwound from one cocoon was about 780 m. This value is about 2.3 times as compared with that of the silkworm reared on the diet adopted in the previous paper, being about 63 per cent of that in the case of the same race reared on mulberry leaves. The number of the eggs laid by one moth was 333. This value is 1.5 times that obtained with the first artificial food. These facts suggest that by improving the composition of the artificial food, results on the silkworm raising and on the cocoon fibres did well. However, the results which are obtained at present with the artificial food is far behind those obtained with fresh mulberry leaves. Ito etc. (1960) reared silkworms on an artificial food whose composition consists of leaf powder, potatoe starch, sucrose, raw soy bean powder and water, but the amount of cocoon fibres produced by silkworm reared on this diet was not over 50 mg per worm. By improving the composition of the artificial food and silkworm raising method and also by finding out silkworms which have a taste for artificial food, it may be possible for silkworm to produce a great amount of cocoon fibres and eggs. The present artificial food should be regarded as a milestone on the road of the achievement of our purpose.

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SUMMARY

Attempts to rear silkworms, *Bombyx mori*, on an artificial food have been made for a long time. However, there are no reports that silkworm larvae are reared only on artificial food from hatching to spinning. Recently the authors reared silkworms only on artificial food which consists of powdered mulberry leaves, potatoe starch, a powder of parched soy bean, frozen soy bean-curds and sucrose, and succeeded in making silkworm larvae produce 36 cocoons, starting from 110 worms hatched from eggs. The silkworms went to the pupae, and the fertilized moth laid their eggs.

RIASSUNTO

Bachi da seta allevati con cibo artificiale.

Per lungo tempo sono stati fatti sforzi per allevare le larve di *Bombyx mori* con cibo artificiale. Non risulta che larve di bachi da seta siano state allevate soltanto con cibo artificiale dalla nascita alla filatura del bozzolo. Ultimamente gli autori hanno allevato bachi da seta soltanto con cibo artificiale consistente in polvere di foglie di gelso, amido di patata, polvere di soia secca, una mistura congelata di soia e di saccarosio, e riuscirono a far sì che larve di bachi da seta producessero 36 bozzoli, partendo da 110 larve neonate. I bachi da seta arrivarono a diventare pupe e l'adulto fertilizzato depose le uova.

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PHENOLIC ACID METABOLITES IN ARTHROPODA

Except for tyrosinase little systematic study has been done on the metabolism of aromatic amino acids in arthropoda from the enzymatic point of view (1). The possible metabolic pathways illustrated by fig. 1, are based on postulated schemes in mammalia (2) and bacteria (3), on experiments with artificial systems, e.g. non specific hydroxylation, or on the occurence of aromatic amino acid derivatives in a number of insects and crustacea. The species we have studied or restudied are underlined and are reported in fig. 1 together with the substances identified. Details on extraction methods and chromatographic identification have been published before (4).

The indole ring of tryptophane could be opened by a peroxidase, leading to kynurenine derivatives. The localisation of hydroperoxidases is discussed further in a paper by Decleir et al. (5). The contribution of 3-hydroxykynurenine to pigmentation is well known; [cfr. e.g. Kikkawa (6)]. A few branch paths are known in silkworm (6) producing anthranilic acid, 3-hydroxyanthranilic acid and alanin. The anthranilic acid derivatives are conjugated with glycine. Kynurenine and kynurenine derivatives might interfere with the quinone tanning process. Indeed Glassman (7) has shown that these products react with quinones formed by tyrosinase activity leading to production of pigments. The metabolic branch leading to 5-hydroxytryptamine, a typical wasp poison, is described by Pavan (8). Kynurenic acid is not found.

Phenylalanine is an essential amino acid for a number of insects (9). We have never been able to identify precursors as e.g. shikimic or prephenic acid. We could never demonstrate synthesis of phenylalanine in crude extracts of *Tenebrio molitor*.

The experiments of one of us (Aerts F.) did not confirm the conclusions of Dennell (10) on non specific hydroxylation leading to hydroquinone, catechol, pyrogallol and to 2,3- and 2,5-hydroxy-derivatives of phenylalanine. The way benzoquinone derivatives are formed is obscure (8).

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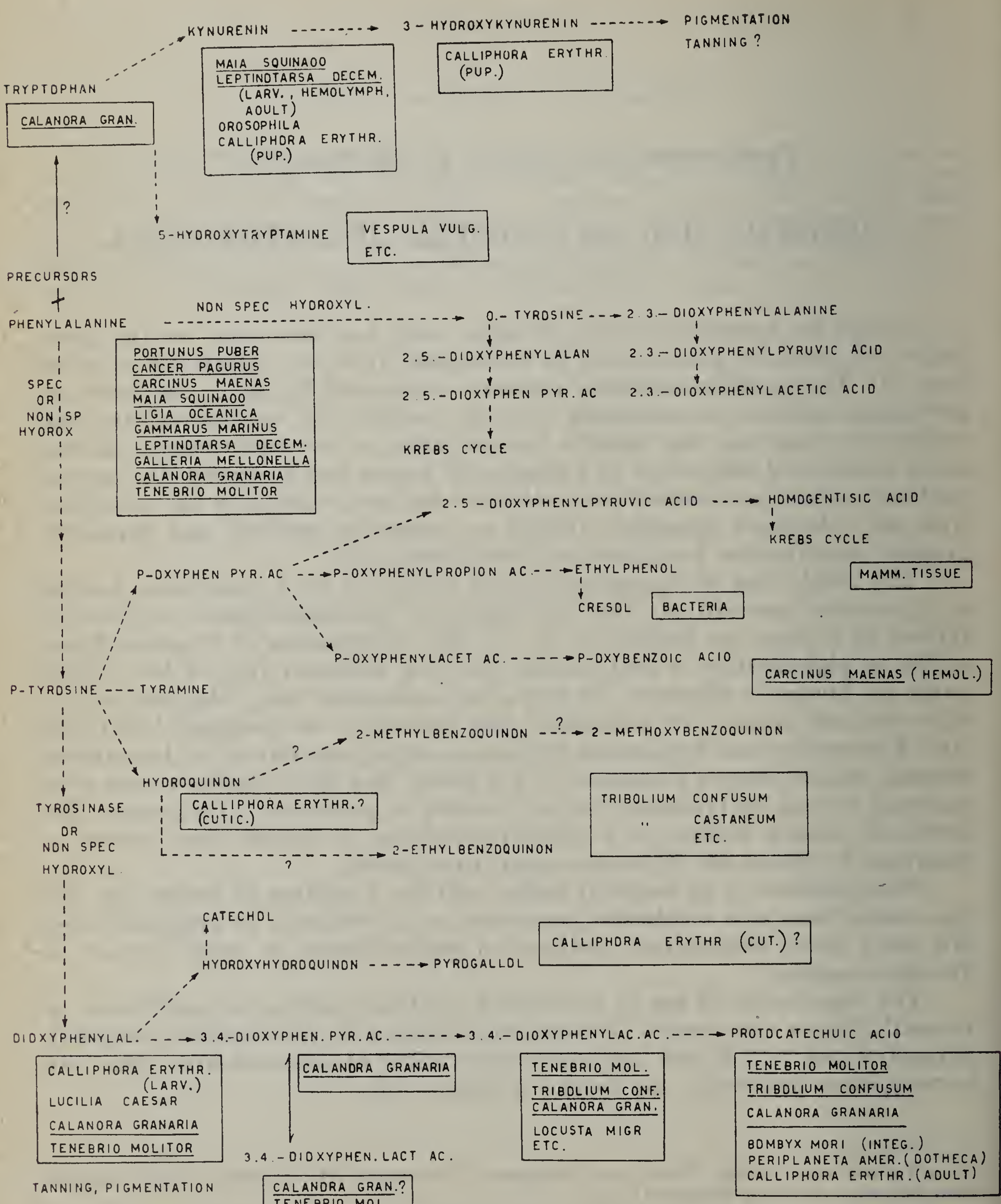
TRYPTOPHAN
CALANDRA GRAN.
KYNURENIN
MAIA SQUINADO
LEPTINOTARSA DECEM. (LARV., HEMOLYMPH, ADULT)
DROSOPHILA
CALLIPHORA ERYTHR. (PUP.)
3-HYDROXYKYNURENIN
CALLIPHORA ERYTHR. (PUP.)
PIGMENTATION
TANNING ?
5-HYDROXYTRYPTAMINE
VESPULA VULG. ETC.
PRECURSORS
PHENYLALANINE
NON SPEC HYDROXYL.
PORTUNUS PUBER
CANCER PAGURUS
CARCINUS MAENAS
MAIA SQUINADO
LIGIA OCEANICA
GAMMARUS MARINUS
LEPTINOTARSA DECEM.
GALLERIA MELLONELLA
CALANDRA GRANARIA
TENEBRIO MOLITOR
SPEC OR NON SP HYDROX
O.-TYROSINE
2.3.-DIOXYPHENYLALANINE
2.5.-DIOXYPHENYLALAN
2.3.-DIOXYPHENYLPYRUVIC ACID
2.5.-DIOXYPHEN PYR. AC
2.3.-DIOXYPHENYLACETIC ACID
KREBS CYCLE
2.5-DIOXYPHENYLPYRUVIC ACID
HOMOGENTISIC ACID
KREBS CYCLE
MAMM. TISSUE
P-OXYPHEN PYR. AC
P-OXYPHENYLPROPION AC.
ETHYLPHENOL
CRESOL
BACTERIA
P-OXYPHENYLACET AC.
P-OXYBENZOIC ACID
CARCINUS MAENAS (HEMOL.)
P-TYROSINE
TYRAMINE
2-METHYLBENZOQUINON
2-METHOXYBENZOQUINON
HYDROQUINON
CALLIPHORA ERYTHR.? (CUTIC.)
TRIBOLIUM CONFUSUM
" CASTANEUM
ETC.
2-ETHYLBENZOQUINON
TYROSINASE OR NON SPEC HYDROXYL.
CATECHOL
HYDROXYHYDROQUINON
PYROGALLOL
CALLIPHORA ERYTHR (CUT.) ?
DIOXYPHENYLAL.
3.4.-DIOXYPHEN. PYR. AC.
3.4.-DIOXYPHENYLAC. AC.
PROTOCATECHUIC ACID
CALLIPHORA ERYTHR. (LARV.)
LUCILIA CAESAR
CALANDRA GRANARIA
TENEBRIO MOLITOR
CALANDRA GRANARIA
TENEBRIO MOL.
TRIBOLIUM CONF.
CALANDRA GRAN.
LOCUSTA MIGR
ETC.
TENEBRIO MOLITOR
TRIBOLIUM CONFUSUM
CALANDRA GRANARIA
BOMBYX MORI (INTEG.)
PERIPLANETA AMER. (OOTHECA)
CALLIPHORA ERYTHR. (ADULT)
3.4.-DIOXYPHEN. LACT AC.
CALANDRA GRAN.?
TENEBRIO MOL.
TANNING, PIGMENTATION

Fig. 1.

The pathway from dopa to protocatechuic acid, suggested by Pryor et al. (11), seems to be very wide spread. Discussion on this subject is formulated in the paper by Aerts et al. (12).

Some of the to day's theories may be fated to evaporate in the light of better knowledge. We would in fact call attention to non identified phenols which do not fit into the schemes presented here. They occur in nearly all species we examined. Aqeous extracts of adult Calandra granaria for instance, contain rather large amonuts of an o-diphenol reacting positively with Evans' and Folin's reagent, with diazotized paranitroaniline, o-dianizidine and sulfanilic acid. The R_F is 0.50 in n-butanolacetic acid- water 4/1/1 and 0.55 in m-e-ketone-water-formic acid-acetone 80/12/2/4. It is a good substrate for *Tenebrio molitor* tyrosinase. On this account it should have one OH-group in para position to the side chain. Positive Folin- and Evans reactions point to a second OH -in o-position (o-diphenol). A spectral shift in the U.V. -range from 278 to 344 mμ, produced by alkalinisation to pH 9.5 could be due to the presence of a CO-group on the nucleus and in p-position to a aromatic OH-group. A free carboxylgroup is excluded. The compound is readily hydrolized by I N HCl or I N NaOH (in a N_2-atmosphere) at room temperature giving rise to two more unknown diphenols and an inorganic acid (probably phosphoric acid).

A second remark should be made about the state of binding of phenolic acids in insect tissues. A number of substances (e. g. unknown compound R 0.50, 0.50, protocatechuic acid and 3,4-dioxyphenylacetic acid) are strongly bound to the debris fraction of the homogenate of *Calandra granaria.* After exhaustive extraction of those compounds by cold water (up to 10 washings) an additional large amount can be liberated by heating (100° C) for 10 min. β-glucuronidase, saturated ureum or boiling ethylalcohol does not produce the same effect. The above mentioned experiments point to the fact that apart from free phenolderivatives and their reaction products fixed on tanned proteins or polymerized to pigments, another class of bound o- diphenols exists, the physiological control of which might be significant.

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SUMMARY

Preparatory to work on aromatic aminoacid metabolism in *Arthropoda* the occurence of derivatives has been studied by paper chromatography. The data obtained with marine crabs, with an isopode, with an amphipode and with various insects were arranged into a table showing possible metabolic relations.

An attempt has been made to follow the pattern of acetone extractable phenols during maturation. The water insoluble fraction of the insect homogenate liberates large amounts of phenolic substances upon boiling or prolonged incubation at 37° C.

RIASSUNTO

Metaboliti di acido fenolico in Artropodi.

Come lavoro preparatorio sul metabolismo di aminoacidi aromatici in Artropodi la presenza di derivati è stata studiata per mezzo di cromatografia su carta. I risultati ottenuti con granchi marini, con un Isopode, con un Anfipode e con vari Insetti, sono riuniti in una tabella mostrante le possibili relazioni metaboliche.

Un tentativo è stato fatto per seguire il campione di fenoli estraibili in acetone durante la maturazione. La frazione insolubile in acqua dell'animale omogenato libera grande quantità di sostanze fenoliche sopra il punto di ebollizione o con un'incubazione prolungata a 37° C.

AERTS F., VERCAUTEREN R., DECLEIR W. (*)

ENZYMES IN THE METABOLISM OF PHENOLIC ACIDS IN INSECTS

It is known from different investigations on the phenolic acids occuring in insects (4, 5), that 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenyllactic acid are present in the adults of *Tenebrio molitor*. As large amounts of phenylalanine and tyrosine are present in the larvae and in the pupae, we can consider them as the precursors of the above-mentioned phenols in the adults.

The possible pathways of their formation is given in fig. 1.

The side chain of tyrosine can be attacked in two ways:

1) by a transamination or by a deamination p-hydroxyphenylpyruvic acid can be formed;

2) tyrosine can give rise to tyramine by a decarboxylation reaction.

The side chain of tyramine and of p-hydroxyphenylpyruvic acid can be broken down to give the acetic acid derivative. Phenolase can hydroxylate the different p-cresol derivatives. 3,4-dihydroxybenzoic acid can be formed from 3,4-dihydroxyphenylacetic acid.

The formation of p-hydroxyphenylpyruvic acid from tyrosine in homogenates of *Tenebrio molitor* larvae could be demonstrated chromatographically.

A tyramine oxidase activity is found in pupae and in adults at the time of metamorphosis.

One of the main enzymes in that schema is phenolase. Thus, apart from its activity in the formation of quinones and melanins, phenolase is also important in the enzymatic hydroxylation of p-cresol derivatives. In order to have any idea about the substrate specificity, phenolase of *Tenebrio molitor* has been partially purified and different substrates have been tested (fig. 2).

From these experiments results, that:

1) phenolase only catalyses the oxidation of p-cresol derivatives, that is, phenols having the hydroxylgroup in para position to the side chain, and of catechol derivatives, that is, 3,4-dihydroxyphenylcompounds;

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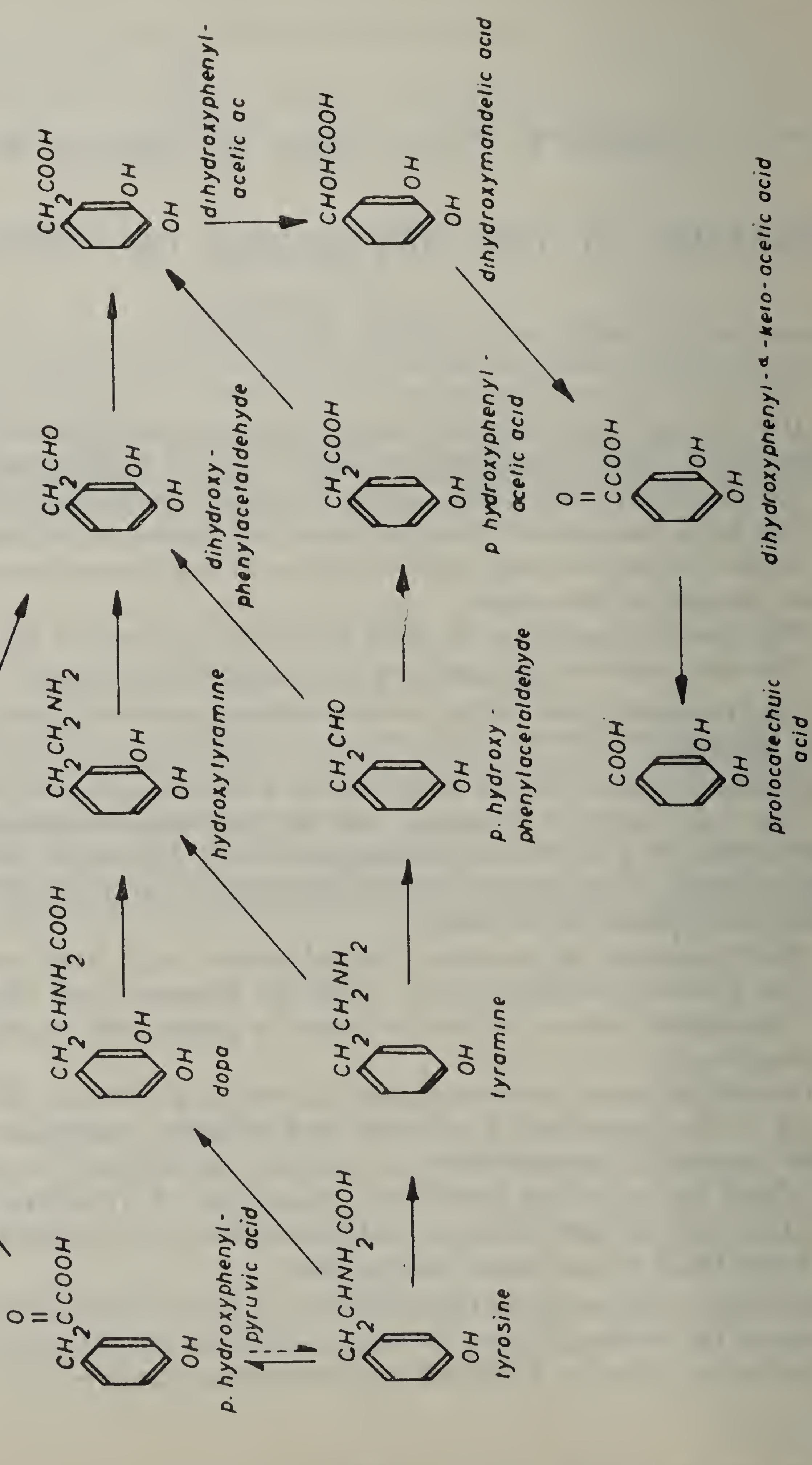

Fig 1 Possible Pathways for the Biosynthesis of 3,4-dihydroxybenzoic acid. 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenyllactic acid in *Tenebrio molitor*

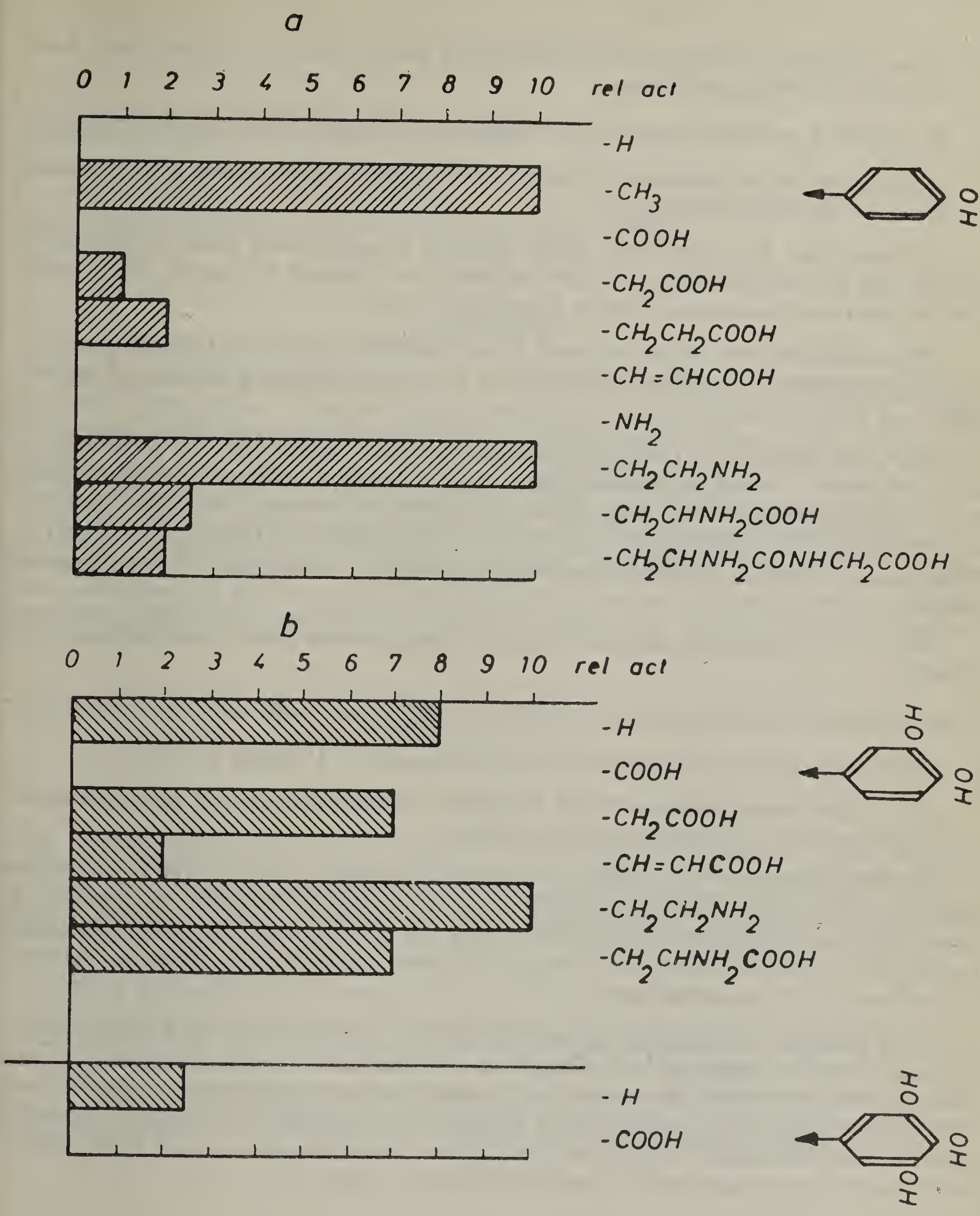

Fig. 2

Relative activity of phenolase from *Tenebrio molitor* on different substrates. The rate of oxygen uptake per minute is taken as a measure for activity.

2 a) activity on p-cresol derivatives compared with the activity on p-cresol.

2 b) activity on catechol derivatives compared with the activity on 3-hydroxytyramine.

2) in the p-cresol derivatives the first carbon atom near the nucleus must bear at least two hydrogen atoms;

3) the nearer a carboxyl group to the nucleus, the lower is the rate of oxidation;

4) the presence of an aminogroup in the side chain seems to have an increasing effect on the oxidation rate.

It must also be mentioned, that dialysed homogenates have a decreased activity on p-cresol derivatives: the activity on tyrosine is largely decreased, that on p-hydroxycinnamic acid is completely lost.

The oxidation rate of caffeic acid being initially very high, falls off quickly to zero; probably phenolase is destroyed by the oxidation products of caffeic acid.

It is also interesting that hydroquinone can be oxidised in the presence of traces of dopa. Probably we deal here with a coupled oxidation. Pryor (4, 5) stated, that protocatechuic acid is the substrate for tanning the insect cuticle. As protocatechuic acid is not oxidised by phenolase of insects, here also a coupled oxidation is probably responsible for the production of the tanning quinone.

For the conversion of phenylalanine to tyrosine two mechanisms are possible:

1) an enzymatic hydroxylation with DPN or TPN and a folic acid derivative (3);

2) a non-enzymatic hydroxylation as put forward by Dennell (1, 2).

We could not demonstrate an enzymatic hydroxylation of phenylalanine in homogenates of *Tenebrio molitor* larvae.

Investigations on the non-enzymatic hydroxylation were carried out with cuticles of *Calliphora erythrocephala* larvae (*). Apart from the formation of traces of tyrosine after an incubation time of a few days, no other phenolic compound could be detected. It could be shown undoubtfully, that no alanine is produced in these conditions.

The processes of hardening and darkening of the cuticle and the production of phenolic compounds in *Tenebrio molitor* take a very short time. Considering this, we think, that in order to study and to separate the different enzyms concerned in the formation of phenolic compounds in *Tenebrio*, investigations must be made with pupae just before metamorphosis and with adults just after it. Investigations in that direction are going on.

(*) These investigations were made at the laboratory of experimental zoology at the University of Manchester. We wish to thank prof. Dennell for his aid and for his kindness.

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SUMMARY

It is commonly accepted that phenolase is active in sclerotization and pigmentation. To clarify the enzyme phase of this process the catalysts are to be isolated, purified and fully characterized. Apart from a system providing the organisms with tanning quinones and melanine a pathway may lead to the production of additional phenolase substrates by breakdown of the phenylalanine side chain or to opening of the aromatic ring.

The substrate specificity of phenolase from *Tenebrio molitor* was investigated.

Specific- or non- specific hydroxylation could not be demonstrated. Phenylalanine is not consumed by crude extracts from *Tenebrio*. One of us has had the opportunity of repeating the experiments at Prof. Dennell's laboratory on hydroxylation in *Calliphora erythrocephala* (last instar larvae). A trace of tyrosine was formed by a heat resistent system after prolonged incubation. Hydroquinon was not recovered. The influence of inhibitors has been studied. The decarboxylation of p-oxyphenylpyruvic acid could be demonstrated.

RIASSUNTO

Enzimi nel metabolismo di acidi fenolici negli Insetti.

E' comunemente accettato che la fenolasi è attiva nella sclerotizzazione e nella pigmentazione. Per chiarire la fase di enzima di questo processo devono essere isolati i catalizzatori, purificati ed interamente caratterizzati. Indipendentemente dal sistema che fornisce gli organismi di chinoni tannanti e melanina, una via può condurre alla produzione di substrati di fenolasi supplementare per rottura della catena dal lato della fenilalanina od all'apertura dell'anello aromatico.

Si studia la specificità del substrato di fenolasi di *Tenebrio molitor*.

Non può essere dimostrata la idrossilazione specifica o non specifica. Fenilalanina non è alterata da estratti grezzi di *Tenebrio*. Uno di noi ha avuto l'opportunità di ripetere gli esperimenti sulla idrossilazione in *Calliphora erythrocephala* nel laboratorio del Prof. Dennel (larve all'ultimo stadio). Una traccia di tirosina fu formata da un sistema resistente al caldo dopo una incubazione prolungata. Non fu scoperto idrochinone. E' stata studiata l'influenza di inibitori. Potrebbe essere dimostrata la decarbossilazione dell'acido p-ossifenilpiruvico.

DECLEIR W., AERTS F., VERCAUTEREN R. (*)

THE LOCALISATION OF POLYPHENOLOXIDASE IN HEMOCYTES

Polyphenoloxidase has been localised in oenocyte-like cells in the hemolymph of *Sarcophaga falculata* (1). The Nadi reagent used is said to be non-specific. We used other substrates to make a critical cytochemical study on the localisation of that enzyme. Investigations are made on hemocytes of *Tenebrio molitor*, *Galleria mellonella* and *Carcinus maenas*.

I. *GALLERIA MELLONELLA*

The hemolymph of *Galleria mellonella* larvae contains oenocyte-like cells, showing a positive phenolase reaction (2). We used tyrosine and p-cresol, catechol, hydroquinone and dopa, pyrogallol and the Nadi reagent as substrates. A leg is cut off and the shed hemolymph is taken up with a Pasteur pipette and spread carefully on a slide. After a 10 min. fixation in formaldehyde vapours the smear is incubated in a mixture of I vol. substrate 0,159 M., I vol. distilled water, and I vol. veronal acetate buffer pH = 7,2. Only the 3,4-dihydroxyphenylcompounds give a strong reaction, p-hydroxyphenylcompounds being slowly oxidised, p-diphenols not at all. The Nadi reagent gives the same picture as the other substrates. With catechol as substrate there is a very distinct focal diffusion of coloured oxidation products out of the positively reacting cells. This assumes for the exactness of the localisation. As results from incubation experiments purpurogalline does not stain the fixed cell. The best picture is obtained with pyrogallol.

Investigations on the influence of fixatives, temperature, pH and inhibition showed that:

1) only formaldehyde vapours save the enzyme;
2) temperatures higher than 50° C and low pH values destroy the enzyme;
3) The enzyme is completely inhibited with KCN (final concentration 0,001 M) and K-ethylxanthate (final conc. 0,02 M).

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This proves the reaction is enzymatic.

II. *TENEBRIO MOLITOR*

The hemolymph of *Tenebrio molitor* contains oenocyte-like cells having the same properties as those of *Galleria mellonella.*

III. *CARCINUS MAENAS*

Pinhey (3) and later Bhagvat and Richter (4) supposed that in the hemolymph of crustacea decapoda cells carry phenolase. Bodine and Allan (5) demonstrated phenolase in the serum of *Astacus fluviatilis,* partially in the inactive form as protyrosinase. We checked this by a cytochemical study of the blood cells of *Carcinus maenas.* We found a positive phenolase reaction to occur in a certain type of cells. These are large oval cells with many large granules. They are very eosinophilic and show a distinct PAS and Millon reaction.

We used tyrosine and tyramine, catechol, dopa and protocatechuic acid, resorcinol and hydroquinone, pyrogallol and gallic acid as substrates. Following the same method as explained we only found a good reaction with pyrogallol and catechol after a 40 min . and with dopa after a 50 min. incubation. Diffusion of oxidation products does not occur and the reaction was also much slower. This is probably due to the fixation method. Formaldehyde vapours produce an intense clotting of the hemocytes. Therefore we have put blood into a drop of 5 % formaldehyde solution on a slide. We were not sure the reaction is enzymatic because the phenolic solutions employed are already coloured after the time needed for the reaction. There must be reckoned with the possibility of adsorption. Incubations with fresh pyrogallol solutions and with solutions coloured by autooxidation showed that:

1) Pyrogallol is oxidised enzymatically to form a pigment. The higher the pH of the incubation mixture, the intenser is the pigmentation of the cells. At pH 2,3 they are colourless, at pH 5 yellow and at pH 7 brown.

2) The cells stain when incubated with a solution of purpurogalline (autooxidised pyrogallol solution). The intensity of the pigmentation increases with decreasing pH values. At pH 2,3 the cells are brown, at pH 7,3 yellow and at pH 10 they are colourless.

The enzymatic oxidation of pyrogallol can be inhibited by K-ethylxanthate and KCN. After preincubation with these inhibitors no pigmentation was obtained with fresh pyrogallol. Incubation with purpurogalline resulted in a slight yellow staining at pH 7 as mentioned before. Moreover we were able to restore the enzymatic oxidation by adding Cu^{++} ions.

We could not obtain colouration of the cells with catecholmelanine solutions. Thus catecholmelanine must be produced in the cells themselves. This

assumes for the exactness of the localisation of the enzyme. It must be mentioned also, that no peroxidase activity could be demonstrated in the hemocytes of *Carcinus maenas.*

IV. THE OENOCYTES OF *TENEBRIO MOLITOR*

In order to find out if there is any histochemical relationship between the oenocytes, found in clusters close to the fat bodies and the tracheal system, and the oenocyte-like cells found in the insect blood, a general histochemical study was made.

The oenocytes of *Tenebrio* are described morphologically by Roth (6). In the cytoplasm of these very large cells (up to 200 μ in diameter) we find large yellowish brown granules responsible responsible for the characteristic yellow-brown colour of the cells. These granules are coloured by Sudan Black B and toluidine blue. The PAS reaction is strongly positive only in large oval corpuscules. We found them often (but not always) and then they were packed together within the cells or individually between them. The outer layer of the large cells shows a remarkable metachromatic reaction, so that the whole intact cluster of cells seems to be surrounded by a metachromatic zone.

Tests for phenolase are negative. The nucleus shows a peroxidase activity (benzidine method after Prenant-modified). Fresh or fixed cells react equally well. The enzyme is destroyed at 120° C. Cell homogenates of oenocytes also show a negative phenolase and a positive peroxidase and catalase activity. Oenocytes of adults and larvae behave in the same way. Peroxidase activity in oenocytes has been described by Glaser (7).

V. CONCLUSION

I) A critical study has been made on the cytochemical localisation of polyphenoloxidase in the hemocytes of *Tenebrio molitor, Galleria mellonella* and *Carcinus maenas.* The best localisation is obtained by incubating the smears in a mixture containing: 1 vol. pyrogallol 0,159 M, 1 vol. distilled water and 1 vol. veronal acetate buffer pH = 7,2. The enzymatic nature of the reaction and the exactness of the localisation are proved:

a) The factor responsible for the reaction is inactivated by some fixatives, high temperature, low pH and is sensitive to inhibitors.

b) Different substrates give the same localisation.

c) The focal diffusion of coloured oxidation products shows they must be produced within the cells.

d) Purpurogallin, the oxidation product of pyrogallol is withheld by the cell.

II) Phenolase activity could be demonstrated in some hemocytes of *Carcinus maenas.* Here staining is due to the enzymatic oxidation of pyrogallol, but adsorption of purpurogalline produced by spontaneous oxidation of pyrogallol must be taken in account. This is proved by the fact that pigmentation takes place when incubating the cells with purpurogalline, the phenoloxidase being inhibited. No pigmentation could be obtained with catecholmelanine proving in this way catecholmelanine must be produced in the cells themselves.

III) We showed the oenocytes of *Tenebrio molitor* to be histochemically quite different from the oenocytoids.

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SUMMARY

Dennell localized polyphenoloxidase in oenocyte-like cells of *Sarcophaga falculata*. His Nadi-reagent method was critised for non-specificity. A number of other substrates were proved to be useful in enzyme localisation. Hemocytes were obtained from several species.

Larval hemolymph cells, described as oenocytoids and characterized by other histochemical tests, were shown to carry a heath labile o-dihydroxyphenolase. The hemolymph enzyme was studied by micro agar gel electrophoresis also. The enzyme reaction was positive for many cells from *Carcinus maenas*, but secondary absorption of oxidation products of pyrogallol had to be taken into account. The true enzymic nature of the reaction was studied on cellular extracts.

RIASSUNTO

Localizzazione di polifenolossidasi in emociti.

Dennel localizzò la polifenolossidasi in cellule simili a enociti di *Sarcophaga falculata*. Il suo metodo Nadi-reagente fu criticato per la non specificità. Altri substrati risultarono utili nella localizzazione di enzimi. Si ottennero emociti di parecchie specie.

Si mostrò che cellule di emolinfe larvali, descritte come enocitoidi e caratterizzate da altri tests istochimici, hanno un o-diidrossifenolasi instabile al calore. L'enzima dell'emolinfa fu anche studiato per microelettroforesi su agar gel. La reazione dell'enzima fu positiva per molte cellule di *Carcinus maenas*, ma si dovette anche tener conto di un assorbimento secondario dei prodotti di ossidazione di pirogallolo. La vera natura enzimica della reazione fu studiata in estratti di cellule.

Sacktor B. (*)

PATHWAYS OF HYDROGEN TRANSPORT IN FLIGHT MUSCLE

The viewpoints of several laboratories on the biochemistry of insect flight muscle have recently been published. These suggest that the subject of metabolism in this tissue has become too broad for brief summarization. Therefore, in this report, a comparatively limited aspect of this topic will be discussed. This concerns the compartmentation of enzymes and coenzymes in the cytoplasm and mitochondria and their interrelationships in biological oxidation and hydrogen transport.

It is known that the sources of energy for the flight muscle are glycogen, trehalose or glucose. Glycogen deposits are found in the cytoplasm of the muscle and the initial reactions of glycogen, trehalose or glucose catabolism are localized in the cytoplasm. During glycolysis the first oxidative step occurs at the triose level and reduced diphosphopyridine nucleotide (DPNH) is formed by glyceraldehyde phosphate dehydrogenase. Now, we have studied the mechanisms whereby this extramitochondrial DPNH becomes oxidized or how hydrogen or reducing equivalents pass through the cytoplasmic-mitochondrial barrier, if indeed, such a barrier exists. By the term, cytoplasmic-mitochondrial barrier, we mean the inability of the enzymes of the intact mitochondria to act on specific substrates outside.

The various pathways which have been described for the oxidation of exogenous DPNH include:

(1) The direct mitochondrial oxidation of exogenous DPNH
$$DPNH + H^+ + 1/2\ O_2 \longrightarrow DPN + H_2O$$

(2) The oxidation of DPNH by the cytoplasmic lactic dehydrogenase
$$DPNH + H^+ + \text{Pyruvate} \rightleftharpoons DPN + \text{Lactate}$$

(3) The oxidation of DPNH by the cytoplasmic α-glycerophosphate dehydrogenase

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DPNH + H^+ + Dihydroxyacetone phosphate $\rightleftharpoons$ DPN + α-glycerophosphate

(4) The oxidation of DPNH by the cytoplasmic malic dehydrogenase
DPNH + H^+ + Oxaloacetate $\rightleftharpoons$ DPN + Malate

The direct mitochondrial oxidation of extramitochondrial DPNH, pathway (1), enables hydrogen to pass through the barrier. In fact, if this mechanism is very rapid then no barrier for DPNH would be evident. Pathway (2) does not permit hydrogen to pass the barrier; instead, it leads to accumulation of lactic acid in the cytoplasmic compartment. On the other hand, pathways (3) and (4), coupled with the respective mitochondrial oxidation of α-glycerophosphate and malate, enable reducing equivalents to pass the cytoplasmic-mitochondrial barrier. These coupled reactions may be illustrated as:

(3 a) DPNH + H^+ + Dihydroxyacetone phosphate $\rightleftharpoons$ DPN + α-glycerophosphate (cytoplasmic enzyme)

(3 b) α-glycerophosphate + 1/2 O_2 $\rightleftharpoons$ Dihydroxyacetone phosphate + H_2O (mitochondrial enzyme)

Sum: DPNH + H^+ + 1/2 O_2 ⟶ DPN + H_2O

Reactions 3a and 3b represent the α-glycerophosphate cycle in which substrates acting as carriers to transport hydrogen effect the complete oxidation of extramitochondrial DPNH. The malate-oxaloacetate cycle is shown as:

(4 a) DPNH + H^+ + Oxaloacetate $\rightleftharpoons$ DPN + Malate (cytoplasmic enzyme)

(4 b) Malate + DPN $\rightleftharpoons$ Oxaloacetate + DPNH + H^+ (mitochondrial enzyme)

In contrast to the α-glycerophosphate scheme in which the equilibria of reactions 3a and 3b favor the formation and subsequent oxidation of α-glycerophosphate, the equilibria of the malate-oxaloacetate reactions both favor the formation of malate.

Studies were initiated in our laboratory to determine the contributions of each of these routes in hydrogen transport. We previously reported the very rapid direct oxidation of exogenous DPNH by *Musca* flight muscle mitochondria washed in physiological saline. Appreciable, although less, DPNH oxidase activity was found when the mitochondria were isolated in isotonic sucrose. More recent experiments attempted to reconstruct the processes taking place in the intact muscle. Flight muscle from *Phormia* was gently and carefully teased and measurements of DPNH oxidation were started within one minute from the time the muscle was removed from the fly. With this mild treatment, there was *no* direct oxidation of DPNH in most experiments. This demonstrates the presence of a formidable barrier to extramitochondrial DPNH.

In other experiments with teased *Phormia* flight muscle, the addition of pyruvate caused no oxidation of DPNH. This confirms previous observations on the insignificance of lactic dehydrogenase in fly flight muscle and accounts for the failure to accumulate lactic acid even after exhausting flight.

In sharp contrast to the veritable absence of pathways (1) and (2), the addition of dihydroxyacetone phosphate to reaction mixtures containing DPNH and flight muscle caused an immediate oxidation of all the DPNH. In further experiments, it was found that in the presence of antimycin A, added to prevent the mitochondrial oxidation of α-glycerophosphate, dihydroxyacetone phosphate brought about a stoichiometric oxidation of exogenous DPNH. According to the reasoning behind the α-glycerophosphate cycle, catalytic quantities of dihydroxyacetone phosphate should oxidize all the extramitochondrial DPNH. Thus, if a small amount of dihydroxyacetone phosphate were added to a reaction containing excess DPNH and teased muscle, α-glycerophosphate would be formed by the cytoplasmic dehydrogenase. The α-glycerophosphate would, in turn, be oxidized by the mitochondrial oxidase thereby regenerating additional dihydroxyacetone phosphate. This dihydroxyacetone phosphate would then be available for further oxidation of the extramitochondrial DPNH. Actual experiments showed that catalytic quantities of dihydroxyacetone phosphate, in the absence of antimycin A, caused the oxidation of all the extramitochondrial DPNH. These data provide an *in vitro* demonstration of the α-glycerophosphate cycle.

In teased flight muscle of *Phormia*, it was found that in some experiments oxaloacetate addition caused an oxidation of extramitochondrial DPNH at a rate about 25 per cent that obtained with dihydroxyacetone phosphate. However, it was noted that the rate of DPNH oxidation by malic dehydrogenase was markedly influenced by the concentrations of coenzyme and substrate and that any comparison of the activities of this cytoplasmic enzyme with that of the α-glycerophosphate dehydrogenase must take these variables into consideration. It should be pointed out, however, that in earlier experiments it was shown that the rates of oxidation of α-glycerophosphate and malate by fly flight muscle mitochondria are in different orders of magnitude; α-glycerophosphate was oxidized 100 times faster than malate. This would suggest that the malate-oxaloacetate redox reactions have an equalizing function rather than functioning as a constant hydrogen transport in one direction as is the case of the α-glycerophosphate cycle.

In summary, these data suggest that in flight muscle of flies oxidation of the extramitochondrial pool of DPNH is mediated for the most part by the α-glycerophosphate dehydrogenase and that this enzyme in combination with the mitochondrial oxidation of α-glycerophosphate provides the major vehicle for passing hydrogen of DPNH through the cytoplasmic-mitochondrial barrier.

SUMMARY

The pathways whereby extramitochondrial DPNH is oxidized include: (1) The direct mitochondrial oxidation of DPNH; (2) Oxidation by the cytoplasmic lactic dehydrogenase; (3) Oxidation by the cytoplasmic α-glycerophosphate dehydrogenase; and (4) Oxidation by the cytoplasmic malic dehydrogenase. Reactions (3) and (4) are coupled with the mitochondrial oxidation of α-glycerophosphate and malate, respectively. These coupled reactions enable hydrogen to pass through the cytoplasmic-mitochondrial barrier. Scheme (2) does not. The contribution of each of these routes was determined in flight muscle of *Phormia regina*. Reaction (1) and (2) are absent or negligible. Pathway (3) is the most rapid and is compared with the malate-oxaloacetate system.

RIASSUNTO

I modi del trasporto dell'idrogeno nel muscolo del volo.

I modi per cui il DPNH extramitocondriale è ossidato comprendono: (1) la diretta ossidazione mitocondriale del DPNH; (2) ossidazione per mezzo della latto-deidrogenasi citoplasmatica; (3) ossidazione per mezzo dell' alfa-glicerofosfato deidrogenasi citoplasmatica; (4) ossidazione della deidrogenasi malica del citoplasma. Le reazioni (3) e (4) sono accoppiate all'ossidazione mitocondriale rispettivamente dell'alfa-glicerofosfato e malato. Queste reazioni accoppiate danno la possibilità all'idrogeno di passare attraverso la barriera citoplasmico-mitocondriale. Invece lo schema (2) no. La partecipazione di ognuna di queste vie fu determinata nel muscolo del volo di *Phormia regina*. Le reazioni (1) e (2) sono assenti o trascurabili. La via (3) è la più rapida ed è confrontata al sistema malato-ossaloacetato.

YAMAFUJI K. (*)

NITROGEN CYCLE IN SILKWORM TISSUE

Metabolic virogenesis was first demonstrated in silkworms in 1943 by applying hydroxylamine. In pursuing the behaviour of this amine, we discovered six new enzymes which have led to the establishment of a nitrogen cycle.

PREPARATION OF DEHYDROGENASES AND REDUCTASES

To prepare enzymes, digestion organ-freed tissues were extracted with a secondary sodium phosphate solution and the extract was fractionated with acetone.

Tris buffer, NaCl or $NaHCO_3$ is effective for the extraction of reductases, but ineffective for dehydrogenases. Tissues were homogenized with M/10 Na_2HPO_4 and the supernatant was treated with acetone at about — 10° C. The precipitate which was obtained with 33—55 % of acetone was dissolved either in phosphate mixture of pH 7.3 for estimating reductase or in Tris buffer of pH 7.5 for measuring dehydrogenase.

DETERMINATION OF ENZYMES

Enzymatic activities were determined spectrophotometrically by following the change of the absorption of coenzyme, diphosphopyridine nucleotide.

The reducing processes proceed in the presence of flavin adenine dinucleotide (FAD), phosphate buffer and hydrogenated diphosphopyridine nucleotide (DPNH), and their degree was measured by the decrease (—) of — log T at 340 mμ. The dehydrogenation takes place in the coexistence of DPN, FAD and Tris buffer; it was estimated by the increase (+) of — log T. Time was 30 minutes.

RESULTS

It was first fonud that phosphates inhibit enzymatic oxidation. This is illustrated in Fig. 1. When phosphate buffer was added at the point p, the dehydrogenation of, for example, ammonium chloride was stopped ,and the reduction

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was caused in cooperation with flavincoenzyme. In the mixture to which water was added the oxidation proceeded continuously.

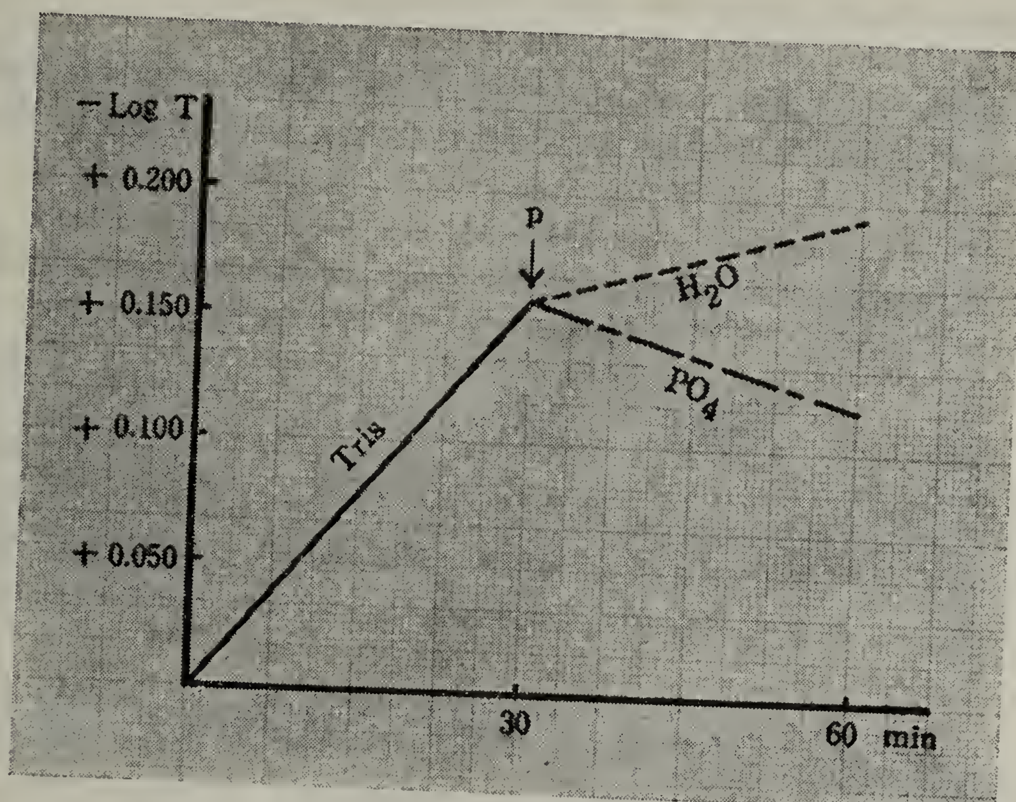


Fig. 1
Effect of phosphate on dehydrogenase and reductase.

The reductase is more heat-labile than the dehydrogenase; it is mostly destroyed by heating at 40° C for 5 minutes. Both groups display the maximum activity at pH 7.5—7.8. No reduction occurs in the solutions containing pyrophosphate, arsenate or borate. In the mixtures having acetate- and veronal buffers, the dehydrogenation happens with the same enzyme preparation.

It was then observed that the coenzymic relations of silkworm enzymes are different from those of liver or yeast ones. As indicated in Table I, the dehydrogenase of insect requires DPN and FAD, while the one of other origins only the former. The reducing system from these three materials needs both coenzymes.

TABLE I

Coenzymes of reductases and dehydrogenases.

	NH_2OH-dehydrogenation, — log T			NH_2OH-reduction, — log T		
	Silkworm	Hen liver	Yeast	Silkworm	Hen liver	Yeast
FAD, 25 γ	— 0.126	— 0,103	— 0.133	+ 0.053	+ 0.060	+ 0.068
FAD, 0 γ	— 0.036	— 0.010	— 0.071	+ 0.013	+ 0.069	+ 0.074

Triphosphopyridine nucleotide (TPN) and flavin mononucleotide (FMN) possess no coenzymic action. In addition, we verified that the enzymatic activities become stronger with increasing amonuts of coenzymes.

It has been believed that there exist three intermediates between ammonia and nitrate. As shown in Table II, each of these substances is reduced or oxidized, indicating that there is an enzymic cycle of inorganic nitrogen.

TABLE II

Reduction and dehydrogenation of inorganic nitrogen.

	$NaNO_3$	$NaNO_2$	$Na_2N_2O_2$	NH_2OH	NH_4Cl	Without substrate
Dehydrogenation, — log T	—0.130	—0.145	—0.141	—0.149	——	—0.040
Reduction, — log T	——	+0.049	+0.061	+0.053	+0.083	—0.007

The concentration of substrates was 2×10^{-4} M in these experiments. Nitrogen salts of higher molarities inhibit the enzymatic action.

DISCUSSION

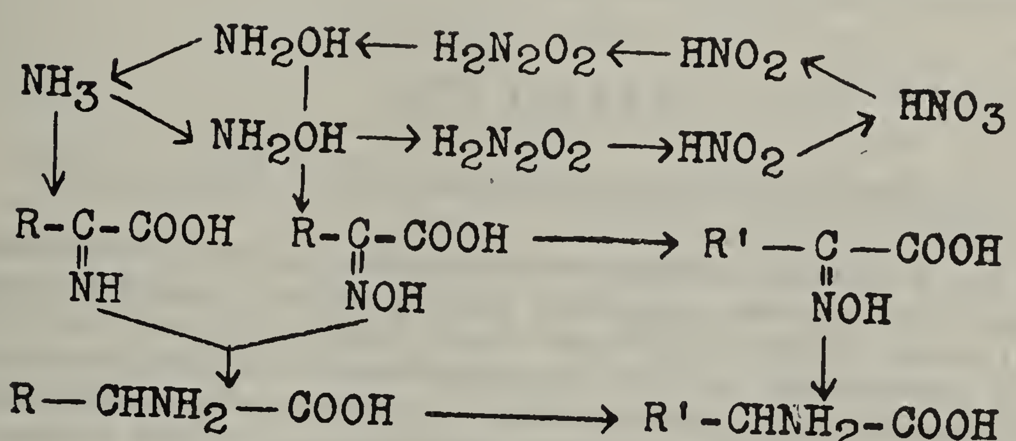
Since the discovery of the virogenic potentiality of hydroxylamine, efforts have been made to prove that this amine appears as an intermediate of nitrogen metabolism in animal bodies. We demonstrated first (1) that oximes which can be formed by spontaneous combination between hydroxylamine and carbonyls are widely distributed in organisms. Two new enzymes, named oximase (2) and transoximase (3), which catalyze the reduction and transfer of oximino group, were then found in some plants and animals, including insect. The possibilty was thus given that hydroxylamine participates in the protein synthesis.

Hydroxylamine is produced by the hydrogenation of nitrates. Although nitrate reductase exists in various vertebrates and silkworms (4), it may be improbable that they contain or take a sufficient amount of nitrates to form oximes. It was desirable to find the way to produce the amine from ammonia which is formed by desamination and other processes in living cells. Although we found about ten years ago (5) that the tissue chyme from Bombyx mori-larvae is capable of oxidizing ammonium salts, the isolation of the oxidase was accomplished only recently (6), based on the finding that the oxidation does not proceed in the solution containing phosphates.

It was further observed (7) that the same enzyme preparation has the ability to dehydrogenate hydroxylamine, hyponitrite or nitrite as well as to hydrogenate nitrate to ammonium. Of late, we succeeded (8) in separating the dehydrogenating system from the hydrogenating one and corrobotated that each

dehydrogenase behaves specifically to different inhibitors. In addition, most of the nitrogenous intermediary products could be determined quantitatively.

The reducing system from nitric acid to ammonia was demonstrated for the first time in animal tissues in the present study. It is uncertain whether the reductases in insects have the same properties as those in microbes which were investigated by Nason (9), Egami (10) and others. Klausmeier and Bard (11) reported on an ammonia dehydrogenase in Bacillus subtilis; its reaction, however, is reversible and ammonium cholride can not serve as the substrate. Here I propose a formula of nitrogen cycle as indicated in Scheme 1. The formulation involves the pathway of amino acid synthesis from ammonia through, for instance, glutamic dehydrogenase-transaminase system by Euler (12) and Braunstein (13) as well as from hydroxylamine through our transoximase-oximase reaction. This general biological cycle has also a close connection with the virus induction by hydrogen peroxide, whose virogenic action was predicted by us (14) about twenty years ago and confirmed by Lwoff (15) in bacteriophages too.



Scheme 1. - Enzymatic nitrogen cycle.

The intermediates, hydroxylamine, hyponitrite, nitrite or oxime, inhibit cellular catalase and cause an accumulation of hydrogen peroxide.

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SUMMARY

Silkworms contain enzyme systems catalyzing cyclic oxidoreduction between ammonium and nitrate. They all require diphosphopyridine nucleotide and flavin adenine dinucleotide as coenzymes. The dehydrogenating group can be separated from the reducing one and each step of dehydrogenation characterized by its behaviour. An enzymatic cycle of inorganic nitrogen and amino acid formation has been established by the discovery of new enzymes, ammonia-, hydroxylamine-, hyponitrite- or nitrite dehydrogenase, oximase and transoximase.

RIASSUNTO

Ciclo dell'azoto nei tessuti del Baco da seta (Bombyx mori).

Nel Baco da seta si trova un sistema enzimatico che catalizza l'ossidoriduzione ciclica fra ammonio e nitrato. Esso richiede nucleotide difosfopiridina e adenina flavina dinucleotide come coenzimi. Il gruppo deidrogenatore può essere separato dal riduttore e ciascuna fase di deidrogenazione è caratterizzata dal loro comportamento. Un ciclo enzimatico dell'azoto e della formazione di aminoacido è stato chiarito per mezzo della scoperta dei nuovi enzimi, ammonio-, idrossilamina-, iponitrito- o nitrito deidrogenasi, ossimasi e transossimasi.

WOLSKY A. (*)

OXIDATIVE METABOLISM AND OMMOCHROME SYNTHESIS IN INSECTS

Ommochrome synthesis is one of the best known chapters of inect chemistry and both biochemists and geneticist have analysed it thoroughly, but until fairly recently no relation was found between the synthesis of these pigments and another biochemical process, the oxidative metabolism of the insect organism. Experiments with *Drosophila* pupae, for example, in which respiratory metabolism was impaired by carbon monoxide, did not reveal any qualitative effect of this treatment on the ommochromes (Wolsky 1937, Rohner and Wolsky 1957, Rohner 1959). However, other material and other techniques have produced by now definitive proof that in certain instances oxidative metabolism and ommochrome synthesis are closely connected and in the following these instances and their implications will be discussed.

The first experimental proof of the connection came from experiments, in which silkworm (*Bombyx mori*) eggs were exposed to centrifugal force shortly after they were deposited (Wolsky 1950). These have shown that the treatment, applied 2 to 3 hours after oviposition, suppresses the formation of the serosa pigment and at the same time reduces considerably the rate of oxygen consumption (see Table I A). The results were confirmed recently in the author's laboratory by Hoffer (1956), who used three different egg stages and a stronger centrifugal force and has shown in certain cases a greatly delayed ommochrome synthesis (see Table I B).

This correlation between respiratory metabolism and ommochrome formation in the silkworm egg has a particularly interesting aspect. Kikkawa (1953) has claimed that the ommochrome synthesis occurs in granular or needle like chromatophores of the cytoplasm, which are the carriers of enzyme aggregates of several biochemical processes and closely resemble mitochondria. Caspari and Richards (1958) have shown that the same cytoplasmic particles are the site of RNA synthesis. Enzymes of oxidative metabolism seem to be also located here and the reduced respiratory metabolism after centrifuging, observed in

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many instances, is generally ascribed to the dislocation of these particles (e. g. Brachet, see Wolsky, 1950). Applying the same explanation to the delayed or suppressed ommochrome synthesis would explain why centrifuging has such strikingly parallel effect on oxidative metabolism and ommochrome synthesis.

TABLE I

A. Effect of centrifuging silkworm eggs with 3000 g for one hour (Wolsky).

Experiment	Oxygen consumption (control = 1.0)	Pigment
Control	1.0	+
Centrifuged 2-3 hrs. after oviposition . . .	0.67	—
Centrifuged 14-16 hrs. after oviposition . .	0.85	+

B. Effect of centrifuging silkworm eggs with 6000 g for 30 minutes (Hoffer).

Experiment	Oxygen consumption (control = 1.0)	Pigment
Control	1.0	+
Centrifuged 2-3 hrs. after oviposition . . .	0.88 (?) (*)	—
Centrifuged 13 hrs. after oviposition . . .	0.71	(+) (**)
Centrifuged 19 hrs. after oviposition . . .	0.74	(+) (***)

(*) Probably « injury respiration ».
(**) Delayed pigmentation (completed in 32 days).
(***) Delayed pigmentation (completed in 25 days).

An even more direct proof of the parallelism between respiration and ommochromes was provided recently by experiments of Rohner (1959, see also Rohner and Wolsky 1957) carried out in the author's laboratory with pupae of the parasitic chalcid wasp *Mormoniella vitripennis*, exposed to carbon monoxide-oxygen mixtures during metamorphosis. This treatment (which is a rather specific inhibitor of the respiratory mechanism, notably the cytochrome system) reduced the rate of development considerably and at the same time suppressed the formation of the dark ommochrome pigment in the compound eyes (Table II). The eyes of *Mormoniella* contain in addition to ommochrome also a red pteridin pigment, which is normally invisible in the adult eye because it is covered up later by the ommochrome. After carbon monoxide treatment however, the red pigment remains visible indefinitely and the treated specimens have bright red, instead of dark brown, eyes. This phenomenon is a bioche-

mical phenocopy as it is an imitation of the phenotype of certain spontaneous, and radiation induced mutations of the species, described by Whiting (1955, 1956) and Kayhart (1956), in which the brown pigment is missing.

Table II

Completion of developmental stages (in days) by *Mormoniella* pupae reared at 25° C. in air and in carbon monoxide-oxygen mixture (9:1) respectively. (After Rohner, modified).

Stages	Air	CO/O_2 (in light)	CO/O_2 (in dark)
White pupa	0	0	0
Amber pupa	1	1	1
Red eye color	2.5	3.5	4.5
Brown eye color	3	—	—
Thorax blackening	4 - 5	6 - 7	8 - 9
Addomen blackening . . .	6	8	10
Hatching	7	—	—

It would have been hard to explain the parallelism between respiratory metabolism and ommochrome formation a few years ago. Today, however, information is available which makes it possible to give the phenomenon a formal explanation. It seems that there is a common factor in the two processes: various quinones, which act as intermediary links both in respiratory metabolism and in ommochrome formation. The studies of Martius (1954) have shown that vitamin K (phylloquinone) acts as an intermediary link between DPN and cytochrome b in the respiratory chain, while Karlson and Wecker (1955) have suggested (on the basis of their studies of tyrosinase activity in insects) that dopa and dopa-quinone — intermediary steps in melanin formation — may have a similar role in the respiratory metabolism of insects. Perhaps the most interesting finding in this connection is that of Heller and Szarkowska (1956), who claim that in some insects, notably in the moth *Celerio euphorbiae*, quinones can entirely replace oxygen as terminal H-acceptor and can produce a kind of « quinone respiration » when the cytochrome system is blocked by KCN.

Quinones seem to play an equally vital role in ommochrome synthesis, and again dopa-quinone is suspected in the first place. Butenandt, Biekert and Linzen (1956), who have clarified the final steps in the formation of one kind of ommochrome, xanthommatin (a phenoxazon pigment) maintain that these steps (which consist of the condensation of two 3-hydroxykynurenin molecules under release of some 8 H atoms) need the presence of powerful H acceptors and in vivo dopa-quinone seems to act as such. Glass (1957) on the other hand

suggests (on the basis of studies on genes, which affect both ommochrome and melanin) that dopa-quinone and hydroxykynurenin form a chemical compound, an« amino quinone », which is the brown ommochrome pigment. The explanation of Butenandt's group is better supported by facts than that of Glass, but it is significant that both explanations are based on the role of quinones.

Thus there seems to be a certain competition for these substances between respiration and ommochrome formation. Although in many cases there seems to be a sufficient differentiation of the enzyme reactions of the two processes to prevent interference and overlapping, there are instances, in which a common pool of quinones is utilized by both processes. In such instances it can happen that a partial or total inhibition of one enzyme system (e. g. inhibition of the normal oxidative metabolism by CO or KCN) will result in a greater demand on the quinone resources (to maintain the essential function — in the given example respiration — by emergency measures) and the other process (in the given example ommochrome synthesis) will suffer the consequences of the resulting shortage, as in the cases reported here.

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SUMMARY

Ommochromes are dark or yellow pigments, formed from tryptophane through kynurenin and deposited mostly in insect eyes, but sometimes elsewhere (e. g. silkworm egg membrane - Kikkawa). The author and co-workers (Hoffer, Rohner) have presented evidence that in certain instances depression of oxidative (respiratory) metabolism has an inhibitory effect on ommochrome synthesis. Centrifuging silkworm eggs during the first day of development reduces oxidative metabolism and suppresses ommochrome formation. Treating pupae of the parasitic chalcid wasp Mormoniella during metamorphosis with carbon monoxide has the same double effect. The significance of these cases and a tentative explanation of their biochemical mechanism are discussed.

RIASSUNTO

Metabolismo ossidativo e sintesi degli ommocromi negli Insetti.

Gli ommocromi sono pigmenti scuri o gialli formati dal triptofano attraverso la kinurenina e depositati soprattutto negli occhi degli Insetti, ma a volte anche altrove [per esempio nella membrana dell'uovo del baco da seta (Kikkawa)]. L'autore e i collaboratori (Hoffer, Rohner) hanno dimostrato che in alcuni casi l'abbassamento del metabolismo ossidativo (respiratorio) ha un effetto inibitore sulla sintesi dell'ommocromo. Centrifugando uova di bachi da seta durante il primo giorno di sviluppo si riduce il metabolismo ossidativo e si sopprime la formazione dell'ommocromo. Trattando pupe dell'Imenottero Calcidide parassita *Mormoniella* durante la metamorfosi con ossido di carbonio si ha lo stesso doppio effetto. Si discute il significato di questi casi e si tenta di spiegare il loro meccanismo biochimico.

LAUFER H. (*)

STUDIES OF CHANGES IN ENZYMATIC ACTIVITIES OF BLOOD PROTEINS IN THE DEVELOPING SILK MOTH

The post-embryonic development of giant silk moths is under endocrine control. The morphological changes that occur during molting and metamorphosis are reflected in alterations in the macromolecular composition of various tissues, the best studied of which is the blood. Telfer (Telfer and Williams, 1953; and Telfer, 1954) in an elegant study described changes in concentration of seven blood proteins in the silk moth, *Hyalophora cecropia*, by means of immunochemistry in agar-gel diffusion. Each of these proteins changes in concentration during the larval-pupal and pupal-adult transformations. However, these experiments did not reveal the sources of these blood proteins, nor their functional significance. The present investigation (1) describes changes detected by zone electrophoresis which occur in the protein constitution during development of a related species of silk moth, *Samia cynthia*, (2) correlates these blood proteins separated by electrophoresis with specific antigens, (3) shows that many of the blood proteins possess enzymatic activity, and (4) reveals the identity of several antigens and enzymes, thus establishing biochemical functions for these proteins. Further experimental procedures indicate (5) the sources, possible sites of synthesis, and sites of utilization of certain of these proteins.

Zone electrophoresis was carried out in starch gels according to the method of Hunter and Markert (1957). Samples were prepared, subjected to electrophoresis, and stained for protein, esterases, and phosphatases, as described by Laufer (1960). Dehydrogenase activity was determined by the method of Nachlas, *et al* (1960). The immunochemical procedures used have already been described (Laufer, 1960).

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Fig. 1-4.

Changes in protein and enzyme patterns during development of *cynthia* revealed by zone electrophoresis in starch gels. Electrophoresis was for 16 hours at 4° C with a voltage drop of 3.5 volts/cm. In all but the MDH assay barbital buffer was used at pH 8.6, 0.02 M. The origin and point of application of the samples is at the bottom of each starch strip. Only the migration toward the anode is shown in these figures.

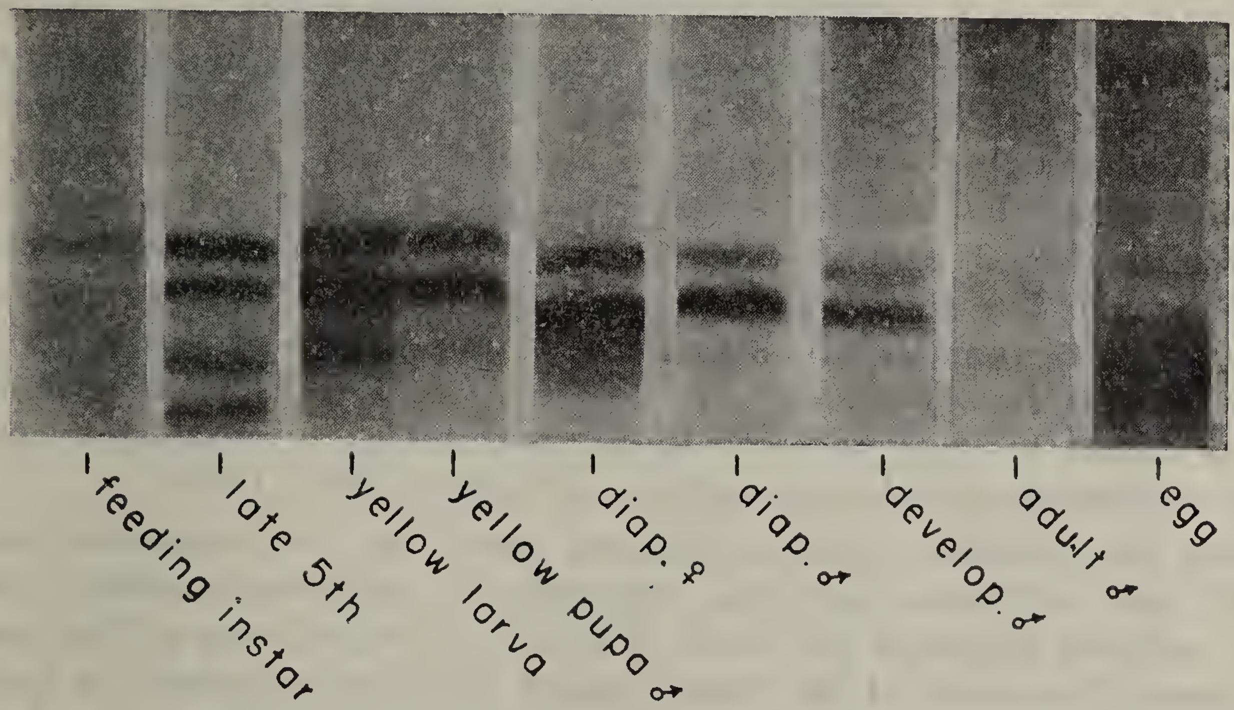

Fig. 1

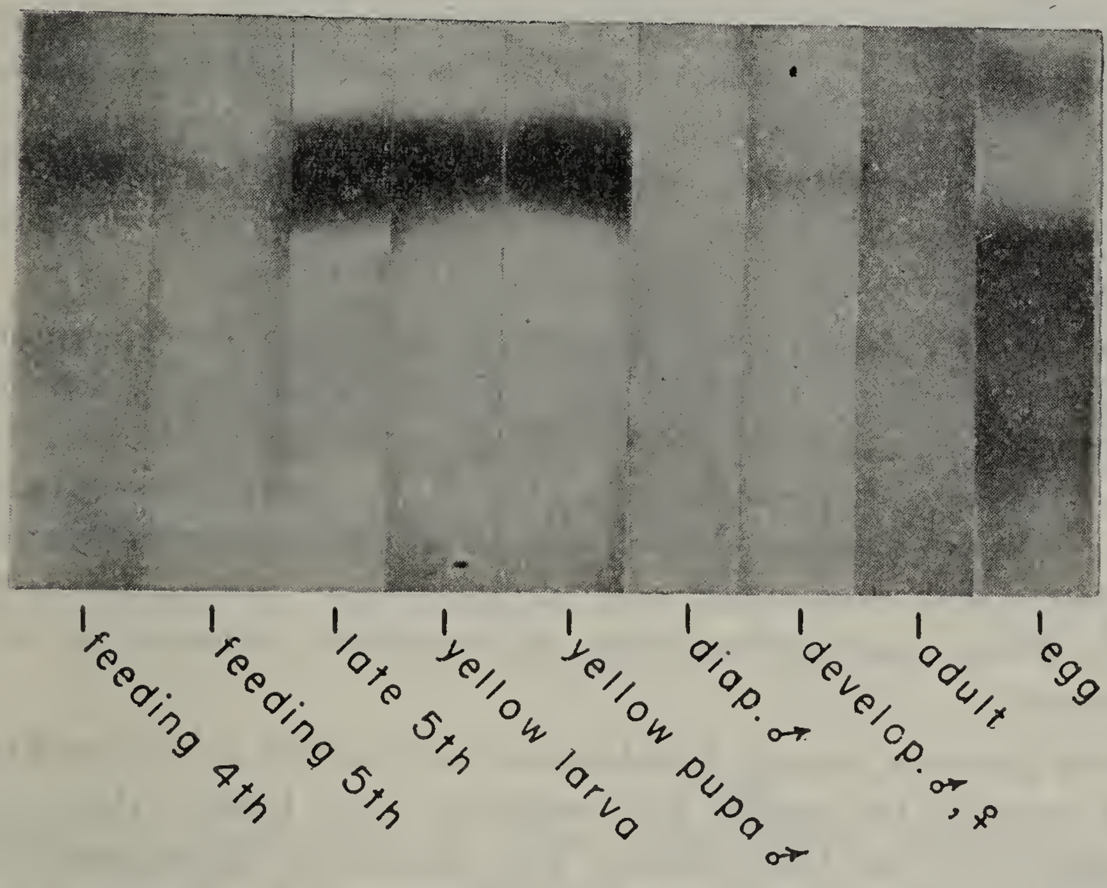

Fig. 2

Fig. 1. Stained for protein with naphthol blue black. The first pattern is of blood from a fourth instar.

Fig. 2. Stained for esterases with alpha-naphthyl butyrate and fast blue bb. The fastest component in the egg extract probably corresponds to the rapidly migrating esterase found in the blood of animals at other stages.

Figure 1 shows the patterns obtained when blood samples of selected stages of *cynthia* were subjected to zone electrophoresis in starch (see also, Laufer 1960). Until the fifth instar larva there is only a low concentration of blood proteins; only one band stains intensely in the fourth instar. However, near the end of the fifth instar, several proteins become more concentrated, only to diminish again with the onset of pupation and diapause. As figure 1 shows, during diapause only two major protein bands remain in the blood of the male, whereas in the female an additional broad band is present. In both sexes the protein concentration increases during development of the adult. But after emergence, in the adult male, all of the blood proteins greatly diminish. By contrast, in the female moth, the blood proteins persist until they become incorporated into the eggs (figure 1). The female-specific protein is present in high concentration in the fat body of females which seems to be the source of this protein. The appearance of this female-protein in eggs suggests a transfer of protein from fat body to blood to eggs, across cellular boundaries, a conclusion consistent with the immunochemical observations of Telfer (1954, 1960).

To determine the relations between protein bands in electrophoretic and antigen-antibody agar diffusion patterns, respectively, immunochemical tests were made with sections of starch gels each of which contained one of the four major protein bands. For each of the bands found by electrophoresis, at least one specific major precipitate band was observed by immunochemical techniques. Furthermore, each of these antigens was well-localized within the starch, indicating that a separation of antigens as well as of proteins had been accomplished by electrophoresis. The correspondence between blood proteins separated by zone electrophoresis and by serum-agar techniques indicates that the electrophoretically separated protein may be identical with specific antigens. Proof of this assertion in the case of the esterases is given below.

When blood proteins of *cynthia* and *cecropia* were tested for enzymatic activity in starch electrophoresis, a large variety of enzyme activities was observed, including a number of esterases (figure 2, as many as eight different esterases being found in *cecropia*), phosphatases (figure 3), carbohydrases, chymotrypsins, tyrosinases, lipases, lactic dehydrogenases, malic dehydrogenases (MDHs, figure 4), isocitric dehydrogenases, and alpha-glycerol phosphate dehydrogenases (α-GPDH). These enzymes undergo characteristic changes in activities during development.

Changes in esterase patterns during development of *cynthia* are as striking as those of the blood protein. In *cynthia* five esterases were found; three migrated toward the anode, two to the cathode. One of these, which migrates rapidly towards the anode, is commonly found in all stages of development, but has greatly reduced activity or was inactive in the blood of diapausing pupae. This « rapid esterase » is reactivated again with the onset of development (figure 2).

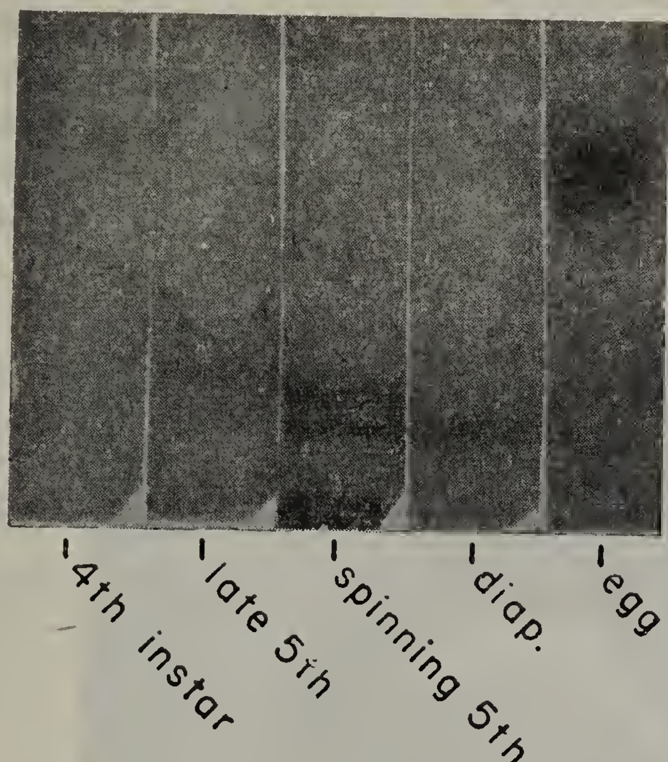

Fig. 3

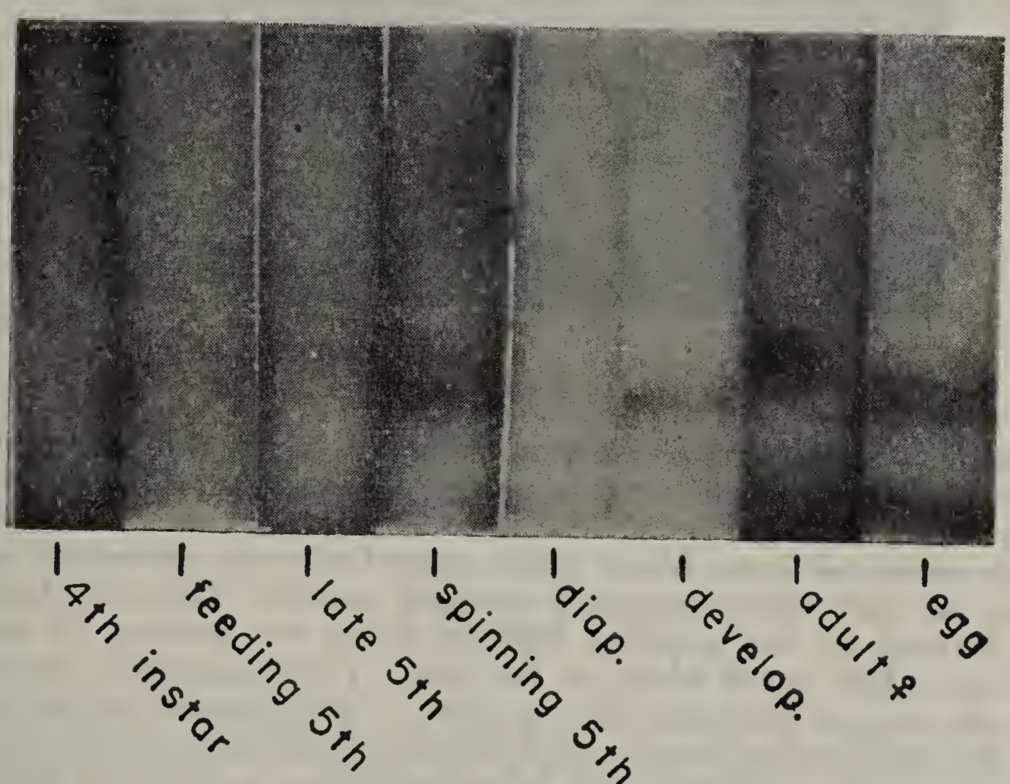

Fig. 4

Fig. 3.

Stained for phosphatase with sodium alpha-naphthyl phosphate. Phosphatase activity in the spinning fifth instar corresponds to the regions of protein bands 1, 2, and 4.

Fig. 4.

Stained for MDH by the procedure of Nachlas, *et al.* Tris buffer 0.02 M, pH 7.0. At pH 8.6 at least two bands with MDH activity were resolved in blood samples from the fifth instar, developing adults, and egg extracts, but not in the diapausing pupa.

Several types of observations suggest that one or more blood esterases are derived from the mid-gut. Extracts of mid-gut contain an esterase identical in electrophoretic mobility with one « rapid esterase » found in the blood. When the blood of a diapausing pupa is diluted with Ringer's solution, the « rapid esterase », which is normally not detectable, is activated. However, when the mid-gut is surgically removed, the « rapid esterase » never appears. But when a mid-gut is transplanted into a mid-gut-less pupa, the enzyme can again be detected in the blood, implicating the mid-gut as an important structure for the synthesis (or at least activation) of this enzyme.

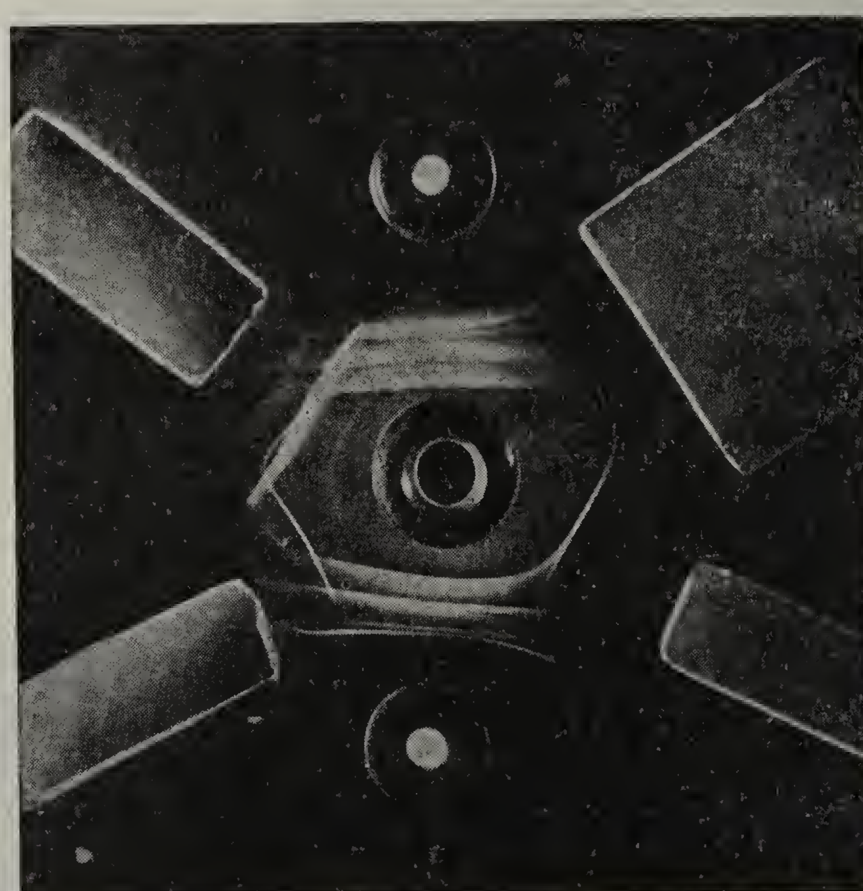

Fig. 5.

Immune precipitates obtained by reacting antiserum to insect blood (hemolymph), in the central reservoir, with insect blood or separated proteins of insect blood resolved by starch electrophoresis. The circular reservoirs at the top and bottom of the figure contained insect blood which reacted with the antiserum by forming a precipitate for each antigen-antibody complex present. The other bands in the agar were formed by the proteins diffusing from the sections of starch after electrophoresis. The starch block at the lower right contained electrophoretic protein 1; the one on the lower left, protein 2; and the one on the upper left, protein 3. For each major protein seen in zone electrophoresis there is a minimum of one specific precipitate seen in agar-diffusion as revealed by the immunochemical test.

With relatively few exceptions (see figures 3 and 4) most of the enzyme activities distinguishable after starch electrophoresis occur in the region of the major protein bands. (The major exceptions are the rapidly migrating esterases.) Also, commonly more than one protein band is enzymatically active with each substrate, e.g. esterases, phosphatases, carbohydrases, lipases, MDHs and α-GPDHs, etc. The activities of many of these enzymes are low during diapause, but increase with the onset of development of the adult (figures 2, 3, and 4).

Finally, histochemical staining of agar-diffusion plates demonstrates that at least two esterases are identical with specific antigens, because in these cases the precipitate bands retain their enzyme activities in spite of the fact that they combined with antibody (see also Laufer, 1960).

From these results we are forced to the rather unexpected conclusion that many, if not all, of the blood proteins are enzymes and doubtless play a strategic role in the economy of the insect.

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SUMMARY

The blood proteins of *Hyalophora cecropia* and *Philosamia cynthia* were followed throughout development by means of zone electrophoresis in starch gels to determine significant changes in quality as well as in quantity. The proteins were also examined for enzymatic activities. These changing blood constituents were correlated with the functions of various organs and tissues, particularly the endocrine glands, which ultimately control development and protein synthesis.

Electrophoresis of blood derived from each stage of development reveals a characteristic and specific pattern of protein bands for each, several of which have enzymatic activity. The various organs also reveal distinct patterns of proteins and enzymes, implying that each component may be synthesized and utilized or broken down independently of the others. Possible functions for a number of blood proteins are suggested by the fact that they posses enzymatic activity such as esterase, phosphatase, carbohydrase, chymotrypsin, tyrosinase, etc. The greatest

activity of these hydrolytic enzymes corresponds with the period of most active development. For example, during pupal diapause certain esterases are undetectable. As many as eight distinct esterases are found in *cecropia* during development of the adult. When the adult emerges, esterase activity again decreases. Furthermore, esterase activity may be experimentally increased during diapause. Increasd concentrations of proteins in the blood are associated in part, with protein synthesis. Other constituents may be released, perhaps by lysis, from existing tissues.

RIASSUNTO

Studi dei cambiamenti nelle attività enzimatiche delle proteine dell'emolinfa durante lo sviluppo dei Lepidotteri Hyalophora cecropia *e* Philosamia cynthia.

Le proteine dell'emolinfa dei Lepidotteri *Hyalophora cecropia* e *Philosamia cynthia* furono seguite per tutto il loro sviluppo per mezzo di elettroforesi in soluzioni di amido allo scopo di determinare significativi mutamenti sia nella qualità che nella quantità. Le proteine furono anche esaminate per le attività enzimatiche. Questi mutevoli costituenti dell'emolinfa furono messi in rapporto con le funzioni dei vari organi e tessuti, in modo particolare con le glandole endocrine che in fine controllano lo sviluppo e la sintesi delle proteine. La elettroforesi dell'emolinfa derivata da ogni stadio di sviluppo, rivela un modello caratteristico e specifico di strisce di proteine, parecchie delle quali hanno attività enzimatica. Inoltre i vari organi rivelano modelli distinti di proteine ed enzimi; il che vuol dire che ogni componente può essere sintetizzato ed utilizzato o infranto indipendentemente dagli altri. Le possibili funzioni per certe proteine dell'emolinfa sono suggerite dal fatto che posseggono attività enzimatica come esterasi, fosfatasi, carboidrasi, chimotripsina, tirosinasi, ecc. La più grande attività di questi enzimi idrolitici corrisponde al periodo dello sviluppo più attivo. Per esempio durante la diapausa pupale alcune esterasi non sono evidenziabili. Otto distinte esterasi si trovano in *cecropia* durante lo sviluppo. Quando l'adulto sfarfalla l'attività dell'esterasi decresce ancora. E ancora, l'attività dell'esterasi può essere aumentata sperimentalmente durante la diapausa. Maggiori concentrazioni di proteine nell'emolinfa sono associate in parte con la sintesi delle proteine. Altri costituenti possono essere liberati dai tessuti esistenti, forse attraverso lisi.

CHEN P. S. (*)

CHANGES IN AMINO ACIDS AND PROTEINS DURING LARVAL DEVELOPMENT OF *DROSOPHILA* AND *CULEX* (1)

During the early larval stages of insect development growth is the dominating phenomenon. However, in the holometabolic forms the morphogenetic processes become more complicated shortly before pupation and at metamorphosis. These processes involve the formation of puparia, the break-down of larval tissues and the building-up of imaginal organsystems. In connection with our biochemical analysis of lethal mutants, metabolic changes in free amino acids and proteins have been investigated both in the body extracts and in the hemolymph at various developmental stages of *Drosophila melanogaster* and *Culex pipiens.* It was found that in both Diptera the contents of free amino acids and blood proteins vary as development proceeds, and that such variations reflect the morphogenetic processes. The present report deals with only the recent results of studies on the protein metabolism at the larval period of these two insects.

The larvae of *Drosophila melanogaster* used in our studies were from a wild stock (« Sevelen ») which has been kept for many generations on the standard corn meal-agar-yeast medium in this laboratory. The *Culex pipiens* stock was an autogenous form and the larvae were fed daily with powdered dog-biscuit. All cultures were maintained at 25° C. The free amino acids were investigated by the two-dimensional paper chromatography and the blood proteins by both paper and starch-gel electrophoresis (for details of the technique see Chen 1956, 1958, 1959). Values of total nitrogen were estimated by the micro-kjeldahl method of Boell and Shen (1954).

1. CHANGES IN FRESH WEIGHT AND TOTAL NITROGEN

As shown in Fig. 1 during the development of *Drosophila* larvae there is a rapid increase of both fresh weight and total N at the period from 48 to 96

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(1) The investigation on *Drosophila melanogaster* was aided by a research grant from the « Karl-Hescheler-Stiftung » and that on *Culex pipiens* by a grant from the « Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung ».

hours after egg-laying. Similar curves have been obtained for *Culex* larvae: growth is quite slow up to the sixth day after hatching, then very rapid in the next 4-5 days, and again somewhat slower near the time of pupation (see Chen 1958, Fig. 1). The values of both fresh weights and total N serve as a basis for comparing the contents of amino acids and proteins at the various developmental stages.

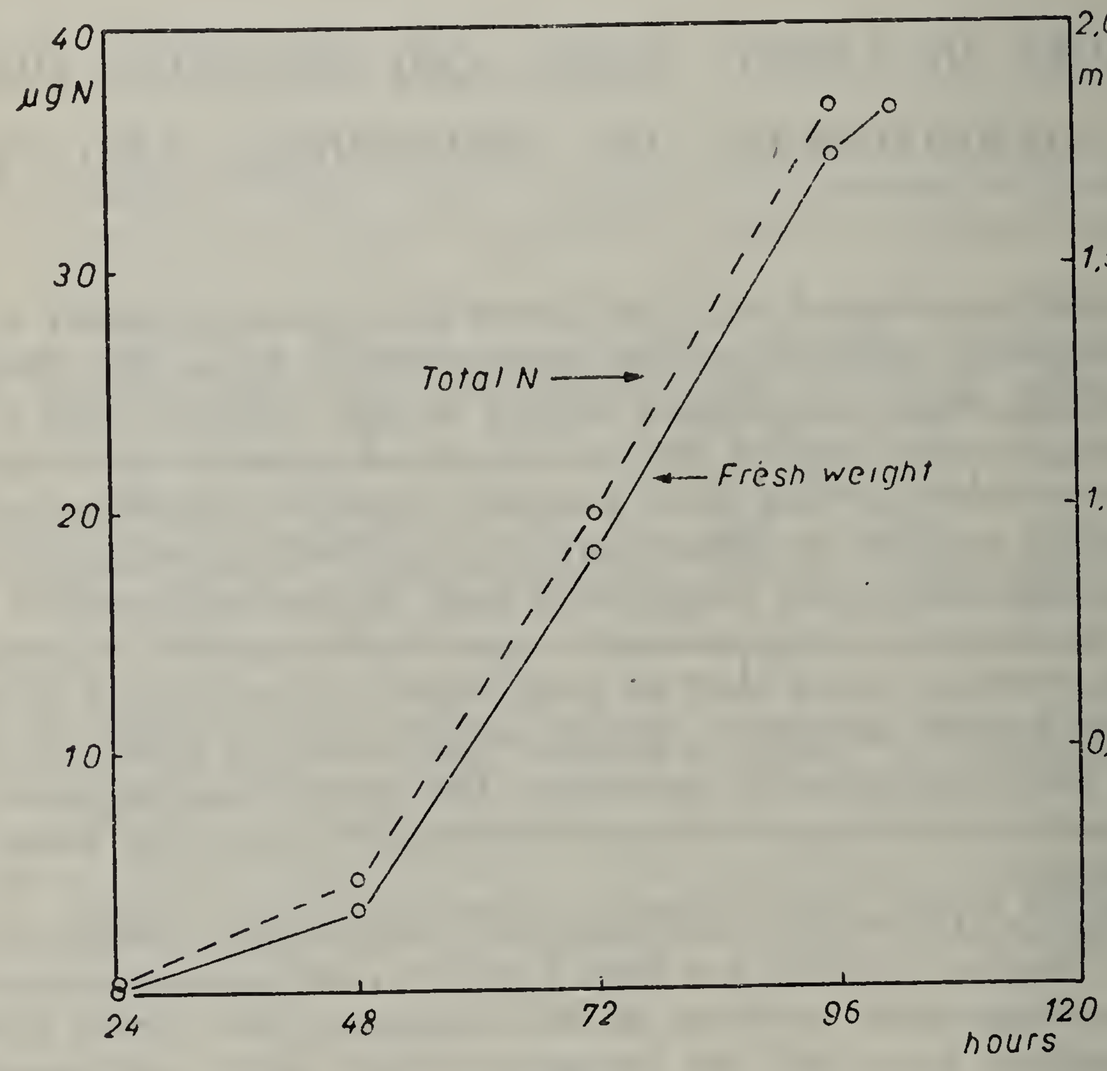


Fig. 1.
Changes in fresh weight (——) and total N (– – –) during larval development of *Drosophila melanogaster* at 25° C. Ordinate: right, fresh weight in mg per larva; left, total N in μg per larva. Abscissa: larval age in hours after egg-laying.

2. FREE AMINO ACIDS

A total of at least 21 free ninhydrin-positive components have been identified on the two-dimensional chromatograms. In the mosquito larvae the total amount of these components per larva increases continuously with the advance of development (Table 1) and increase of size. In *Drosophila* larvae it increases rapidly from 48 to 72 hours, remains constant for the next 24 hours, and then

drops steadily near the time of pupation. If the quantities are expressed per unit body weight, the following differences can be seen: the extinction values of *Drosophila* larvae show a maximum at 72 hours of age, whereas those of *Culex* larvae remain largely unchanged (see the third and seventh column in Table 1). The same result has been obtained by Benz (1955) for *Drosophila*

TABLE 1

Changes in total amounts and total concentrations of free ninhydrin - positive components during larval development of *Drosophila* and *Culex*.

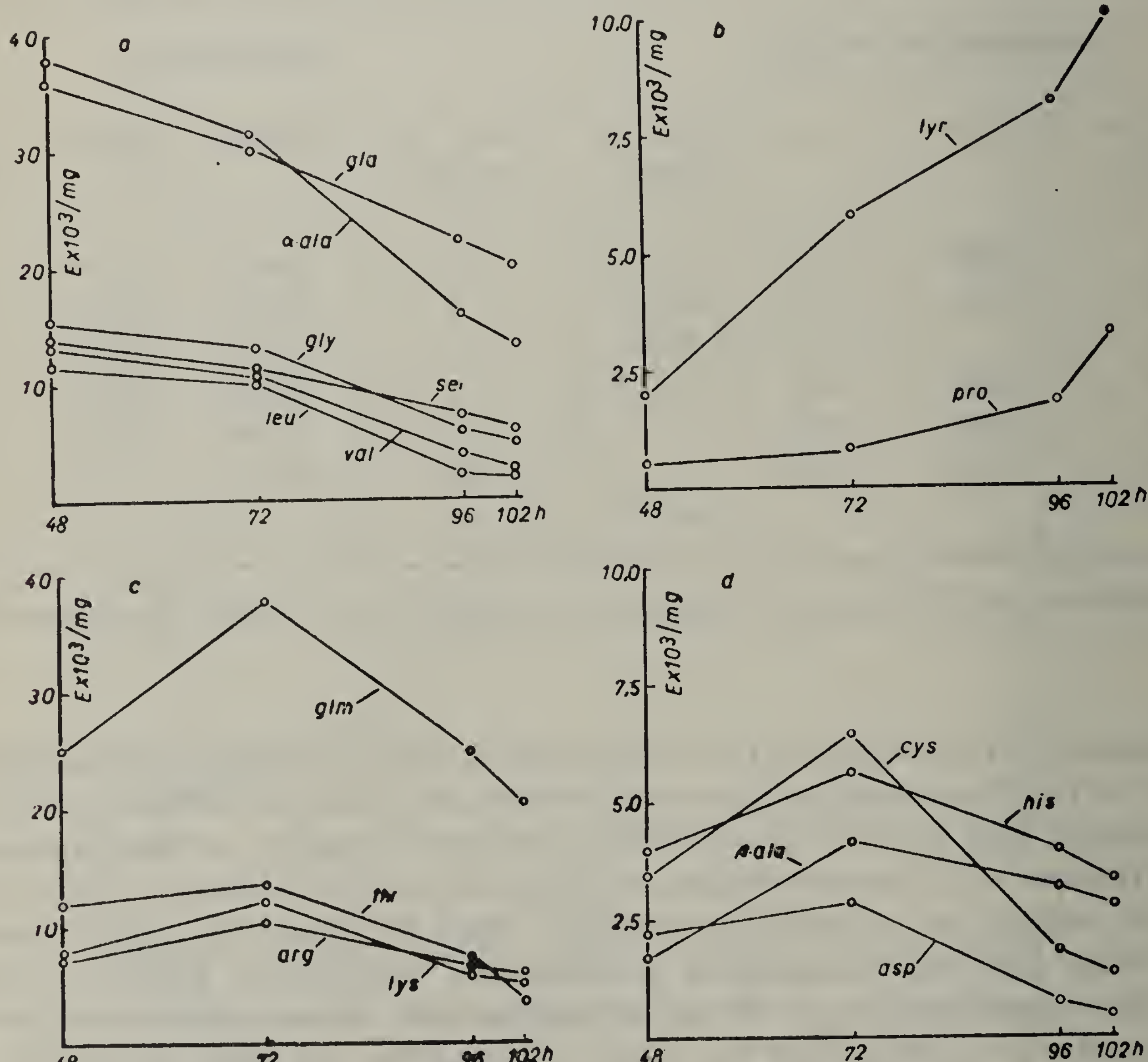
Drosophila melanogaster				*Culex pipiens*			
Larval age in hrs.	E*/larva	E/mg	E/2.6 µl blood	Larval age in days	E/larva	E/mg	E/2 µl blood
48	0.002	0.278	—	5	0.014	0.122	—
72	0.012	0.293	0.595	6	0.039	0.104	—
84	—	—	0.552	7	0.078	0.126	0.210
96	0.012	0.155	0.410	8	0.145	0.124	0.254
102	0.011	0.145	—	9	0.196	0.118	0.218
104	0.007	0.091	—	11	0.461	0.133	0.235
120	—	—	0.404				

* E = Extinction unit. Each figure represents the average value of at least three determinations.

melanogaster. With regard to the total concentration of ninhydrin-positive components in the hemolymph, the two insects also behave entirely differently: in *Drosophila* the blood concentrations decrease rapidly as development proceeds (Hadorn and Stumm-Zollinger 1953, Chen and Hadorn 1954) while in *Culex* the values are essentially constant until the end of their larval life (Chen 1958). For explaining such a difference one has to take the following factors into consideration: 1. It is known that the *Drosophila* larvae leave the culture medium approaching the time of pupation, whereas the *Culex* larvae keep on taking up food. 2. The rate of protein synthesis is not the same in these two insects (see below). 3. In addition to their main function as building blocks of proteins, the amino acids play an important role in the osmoregulation of the body fluid and also serve as sources of energy production through oxidation. Apparently more detailed physiological studies on these two insects are needed in order to give clear answers to the complex problems of amino acid metabolism.

Variations of individual amino acids (extinction per mg fresh weight) for *Drosophila* larvae are presented in Fig. 2. According to their behaviour they can be divided into three groups: Glutamic acid, α-alanine, glycine, serine,

leucine (isoleucine) and valine (methionine) decrease steadily as development proceeds (Fig. 2 a). Glutamine, threonine, arginine, lysine, cystine, histidine, β-alanine and asparagine show a maximum at 72 hours of age (Fig. 2 c, d). In contrast to the above amino acids, tyrosine and proline increase continuously, especially approaching the time of pupation (Fig. 2 b). In a previous study (Chen and Hadorn 1954) it was also found that the blood concentration of tyrosine and proline in *Drosophila* larvae rises rapidly from 72 to 96 hours, while



Changes in free amino acids during larval development of *Drosophila melanogaster* at 25° C. Ordinate: Extinction (E × 10³) per mg fresh weight. Abscissa: larval age in hours after egg-laying.

that of the other amino acids shows a distinct drop during the same period. The same is true for the mosquito larvae: these two amino acids represent the most concentrated ninhydrin-reacting components in their body extracts and reach a maximum shortly before pupation (see Chen 1958, Table 3). This result

is in agreement with the findings of Hackman (1953) that there is a high content of tyrosine and proline in the proteins of insect cuticles. The accumulation of these two amino acids reflects the synthesis of cuticular proteins during the late larval period. It is also known that tyrosine serves as the substrate for the tanning reaction of the larval cuticle at the time of pupation.

3. OTHER FREE NINHYDRIN-POSITIVE COMPONENTS

On the two-dimensional chromatograms of *Drosophila* larvae at least four ninhydrin-positive components which do not correspond to positions of known amino acids have been located. Since they showed several amino acids on acid hydrolysis, these were tentatively designated as peptides (Hadorn and Mitchell 1951, Stumm-Zollinger 1954, Chen and Hadorn 1955, Faulhaber 1959). Very recently it was found that the spots designated as peptide 1 plus peptide 2 contain largely tyrosine-O-phosphate (Mitchell, Chen and Hadorn 1960). This conclusion was reached from both chromatographic and electrophoretic studies on synthetic and natural tyrosine-O-phosphate isolated from larval extracts of *Drosophila*. In *Culex*, ninhydrin-positive spots of similar Rf-values have also been found on the chromatograms (see Chen 1958, Fig. 2). However, it is not yet known if tyrosine-O-phosphate is really present in the mosquito larvae. The physiological function of tyrosine phosphate in the insect is unknown. As in the case of tyrosine sulfate in the vertebrates, it may possibly play a role in blood coagulation. Since it is an energy rich compound, it may also take a part in various metabolic processes.

The spot designated as peptide 3 has a brown color and also contains asparagine. Peptide 4 has a position similar to that of cystine and is most concentrated in extracts of *Drosophila* larvae at 72 hours of age.

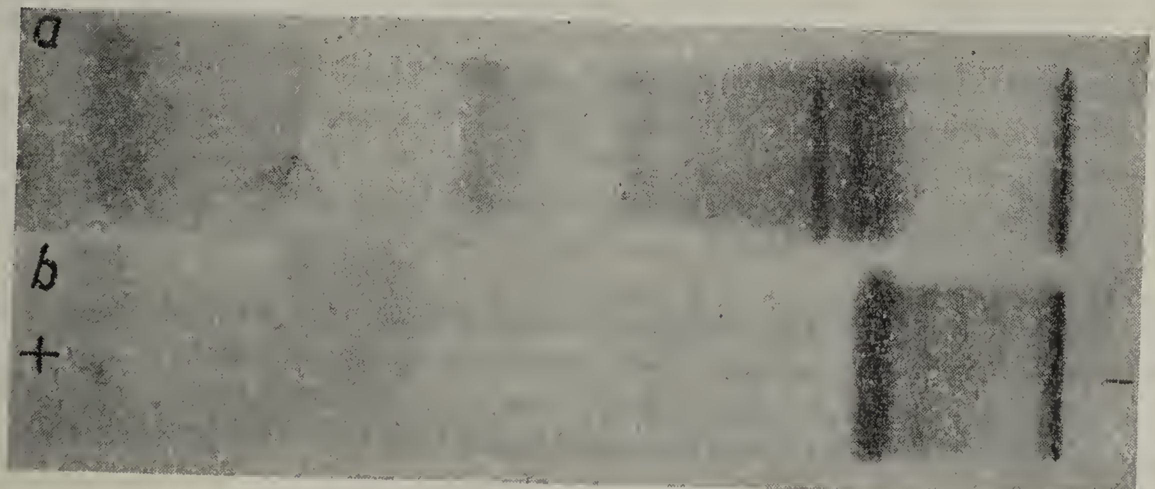


Fig. 3.

Separation of protein fractions in 20 µl larval hemolymph of *Drosophila melanogaster* (a) and *Culex pipiens* (b) by starch-gel electrophoresis.

4. BLOOD PROTEINS

Using paper electrophoresis it was shown that the concentration of blood proteins increases in the course of larval development of both *Drosophila* and *Culex*. The content of proteins per unit volume of hemolymph for *Drosophila* larvae at 96 hours of age is 3,7 times higher than that for individuals aged 72 hours (Chen 1956, Table 1). For *Culex* larvae there is a 4,1-fold increase from 8-9 to 12-14 days after hatching (Chen 1959, Table 1). Recently, using starch-gel electrophoresis at least seven protein fractions have been separated in the hemolymph of *Drosophila* and four fractions in that of *Culex* (Fig. 3). Our preliminary results indicate that the increase of these protein fractions occurs at different rates. For instance, only two fractions are present at the early larval stage of *Culex* and at least four fractions become detectable shortly before pupation begins. A more detailed quantitative analysis of the various fractions is now in progress.

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SUMMARY

Using two-dimensional paper chromatography it was shown that the amount of free tyrosine and proline per unit body weight or per μg total N increases rapidly in the later part of larval development of both insects. Tyrosine-o-phosphate was also found in the blood of *Drosophila*. In *Drosophila* larvae the total concentration of free ninhydrin-positive substances in the hemolymph decreases as development proceeds, whereas in *Culex* it remains constant. By means of starch-gel electrophoresis at least seven protein fractions have been separated in the hemolymph of *Drosophila*, and three fractions in that of *Culex*.

RIASSUNTO

Cambiamenti in aminoacidi e proteine durante lo sviluppo larvale di Drosophila *e* Culex.

Per mezzo di cromatografia su carta bidimensionale si dimostra che la quantià di tirosina e prolina libere per unità di peso del corpo o per microgrammi di N totale aumenta rapidamente nell'ultima parte dello sviluppo larvale di entrambi gli Insetti. Tirosina-o-fosfato fu anche trovata nell'emolinfa di *Drosophila*. Nelle larve di *Drosophila* la concentrazione totale delle sostanze libere ninidrino-positive nell'emolinfa diminuisce mentre lo sviluppo procede; in *Culex* esso rimane costante. Per mezzo di elettroforesi su amidogel almeno sette frazioni di proteina sono state separate nell'emolinfa di *Drosophila* e tre frazioni in quella di *Culex*.

LUCAS F., SHAW J. T. B., SMITH S. G. (*)

THE COMPOSITION OF ARTHROPOD SILK FIBROINS

This paper reports the work we have been doing in the last year or two at the Shirley Institute in the examination of the silks produced by a wide variety of insects and some spiders. Our work in the Silk Department is concerned mainly with an attempt to elucidate the structure of the mulberry silk of commerce produced by *Bombyx mori* but we have been struck, in surveying the field of the silk producing animals, by the wide range of species in the arthropods that make silk. Indeed silk seems to play an important part in the lives of many insects, and of course the importance of various kinds of silk in the lives of spiders needs no emphasis.

We regard as fibroins the protein substances secreted by various species of arthropod in special glands and stored there in solution prior to extrusion to form filaments. Our investigation cannot claim to be a comprehensive study of the field of arthropod fibroins since it has been limited somewhat by the problem of obtaining supplies, and concentrates to some extent on those species that produce relatively large amounts of silk. We have however looked at the silks of over 70 species distributed among some 24 families.

MATERIALS

The families represented in this study are given below; if the silk of more than one species in a family has been examined the number of species is given in brackets: *Chrysopidae, Arctiidae, Cymbidae, Caradrinidae, Lymantriidae* (2), *Saturniidae* (25), *Bombycidae* (5), *Thaumetopoeidae* (6), *Lacosomidae, Nymphalidae, Papilionidae, Galleriidae* (2), *Pyraustidae, Lasiocampidae* (12), *Pyschidae, Cosmopterigidae, Yponomeutidae, Gracillaridae, Plutellidae, Lyonetiidae, Braconidae* (2), *Argiopidae* (2), *Theraphosidae* (3), *Eusparassidae*. The majority of the silks were from the cocoons spun by insect larvae in which to pupate. The *Saturniidae* are perhaps the silk producing family par excellence, but three other families produce substantial amounts of silk – the *Bombycidae, Thaumetopoeidae* and *Lasiocampidae*. There are only a few species in the *Bombycidae* other than the closely related ones of the *Bombyx* genus. The *Thaume-*

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topoeidae contains the *Anaphe* and *Hypsoides* genera that produce large communal cocoons. A few of the species examined produce silk as a web in which the larvae congregate communaly; these were *Thaumetopoea pityocampa*, *Nymphalis io*, *Eriogaster lanestris*, *Euproctis chrysorrhoea*. With the two latter both the web silk and cocoon silk were examined though no fundamental difference was found between them.

While most of the silks examined were from *Lepidoptera*, material has been obtained from two other orders of insects; from two species of *Braconidae* the silk was either cocoon or loose fibre protecting the pupa. From *Chrysopa flava* (*Chrysopidae*) the material was the filamentary egg-stalk; this is produced by the imago of the insect to carry the eggs beneath the leaf of a suitable tree. It differs further from the lepidopterous silks in that it is produced by a different type of gland – the colleterial gland. The larvae of this species also produce a silk in which to pupate but we have not yet been able to obtain a supply of this.

In the field of the spiders we are faced with a far more complex situation since each species produces different types of silk from different glands for different purposes, and we have only been able to examine one or two of these; the silk of the egg cocoon of *Nephila senegalensis* and the nest webs of *Avicularia avicularia* and *Tapinauchenius plumipes*. The silk of *Nephila madagascariensis* was supplied to us reeled and its precise origin is unknown.

METHODS

It is well known that the silk of *Bombyx mori* consists of two proteins, an inner filament of fibroin which is coated with a layer of sericin or silk gum; this latter material serves to cement the fibroin filaments together when these are laid down to form the cocoon. We have only been concerned with the properties of the fibroins and in most cases therefore our silks have been degummed. We have made use of a variety of chemical and enzymic treatments to effect this, the most common involving boiling alkaline solutions. In view of the number of species involved we have not been able to carry out strict control of the extent of degumming; usually a treatment that would cause the filaments to separate has been used and continued for about twice the time that produced the initial separation.

In order to determine their amino acid constituents, all the fibroins were hydrolysed in boiling 6 *N* hydrochloric acid for 24 hours and the acid removed. The hydrolysate was then resolved by two dimensional paper chromatography using butanol/acetic acid/water in the first direction and phenol/water in the second direction. The chromatograms were sprayed with ninhydrin and the intensities of the spots corresponding to the various amino acids were judged by eye. This gave a rough semi-quantitative estimate of the amino acid composition of the fibroins and was applied to all the species. The fibroins of a number of species, however, were analysed quantitatively by one of two methods. In the first, the hydrolysate was treated with 1-fluoro-2:4-dinitrobenzene to

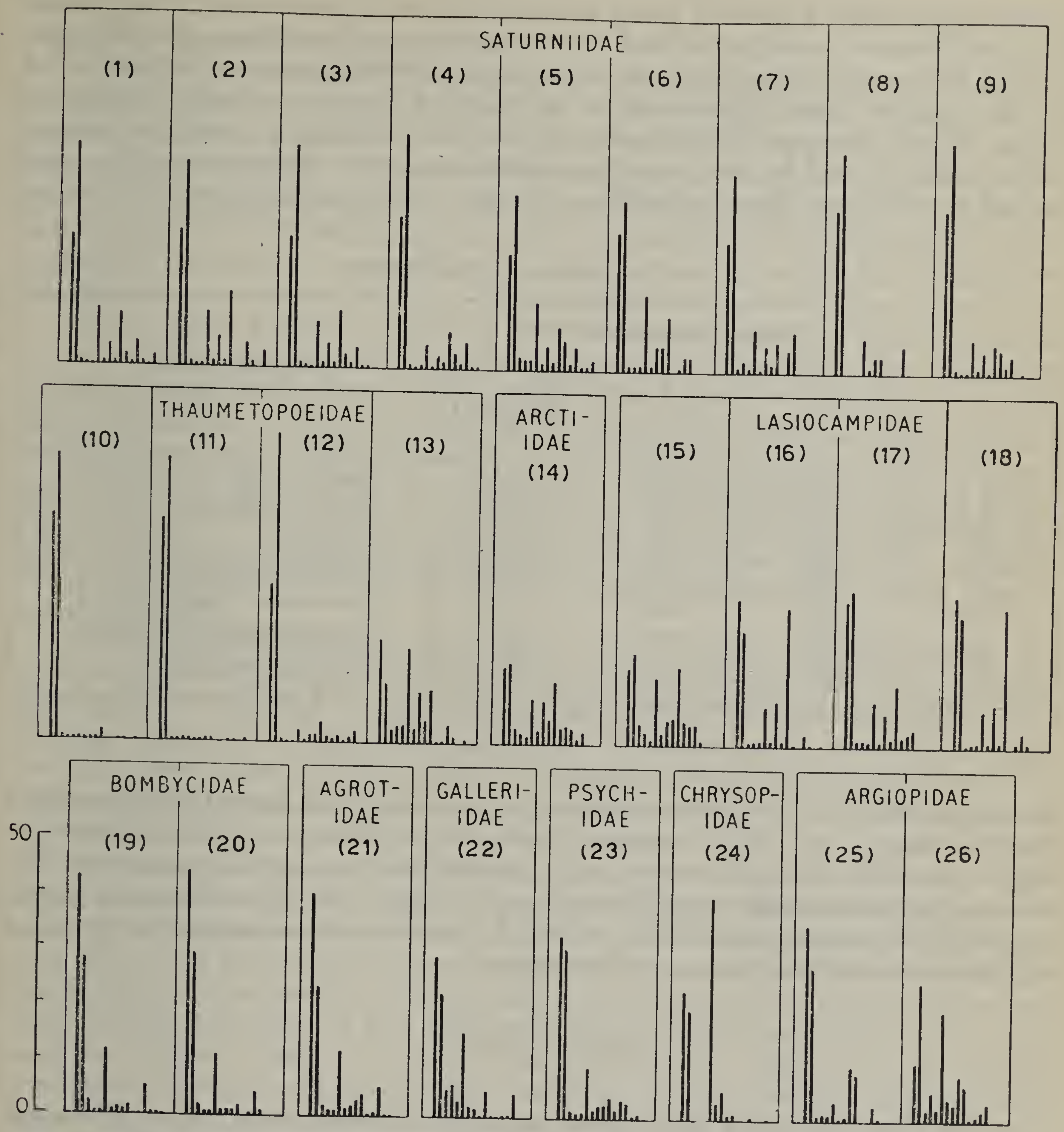
convert the amino-acids to their dinitrophenyl-derivatives which were extracted, separated by chromatography on buffered Celite columns and estimated spectrophotometrically. In the second method the hydrolysate was applied to columns of Zeo-Karb 225 × 8 and eluted with aqueous buffers essentially as described by Spackman, Moore and Stein (1958), the eluate being collected in 2 ml. fractions that were treated with ninhydrin. Measurements of the optical densities of the fractions at 570 mμ, and for proline 440 mμ, enabled the amounts of the separated amino acids to be calculated. The amounts of the amino acids estimated in this way are expressed as amino acid nitrogen per 100 parts of fibroin nitrogen; this indicates the relative numbers of amino acid residues, except for the basic amino acids, arginine, lysine, and histidine whose figures should be divided by 4, 2 and 3 respectively to obtain these values.

The X-ray examination of the fibroins was carried out by Dr. J. O. Warwicker of these laboratories. Bundles of 25 mg of parallel filaments about 25 mm long were used and, when only very small samples were available, the bundle was cemented with gum tragacanth. The vertical bundle was exposed to a horizontal beam of nickel-filtered copper Kα radiation and with the gummed samples was rotated during exposure. The diffraction pattern was recorded on a cylindrical camera of radius 3 cm.

RESULTS

The results of the amino acid analyses are presented for ease of comparison as histograms. Perhaps the first thing to note is that these fibroin filaments are in fact proteins since they all yield amino acids on hydrolysis, and the quantitative results indicate that their total weight in most cases is represented by the amounts of amino acid residues estimated. The striking thing about the types of amino acid that constitute the fibroins is that the simple ones, glycine, alanine, and to a lesser extent serine, predominate. Given this preponderance of the small amino acids, however, there does not seem to be any restriction on the kind of residue that can be incorporated into a fibroin and most of the amino acids have been found, albeit in small amounts in some instances. Cystine appears to occur hardly, if at all, in the fibroins and the highly cross-linked structure that is found in the keratin fibre is therefore excluded. The fibroins from the *Anaphe* and *Hypsoides* genera are remarkable in that alanine and glycine form such a high proportion of their mass. Some 94 % of the fibroin of *Anaphe moloneyi* consists of these two amino acids and the material is largely a polymer of these two amino acids and one of the simplest proteins yet investigated. The fibroin of the egg-stalk of *Chrysopa flava* contains an outstanding amount of serine (40 %), as far as we know higher than in any other protein so far reported.

The X-ray photographs of all the fibroins showed patterns of spots and arcs that indicated that parts of the polypeptide chains are mutually arranged in an ordered way so as to from crystals. From measurements of these arcs



The order of the amino acids in each histogram is: glycine, alanine, valine, leucine, isoleucine, serine, threonine, aspartic acid, glutamic acid, arginine, histidine, lysine, tyrosine, phenylalanine, proline, tryptophane. The ordinate is the amino acid N as a per cent of total N.
The species represented are: (1) *Antheraea pernyi*, (2) *Antheraea mylitta*, (3) *Antheraea yamamai*, (4) *Philosamia cynthia*, (5) *Dictyoploca japonica*, (6) *Cricula andrei*, (7) *Loepa katinka*, (8) *Callosamia promethea*, (9) *Attacus atlas*, (10) *Anaphe moloneyi*, (11) *Anaphe venata*, (12) *Anaphe infracta*, (13) *Thaumetopoea pityocampa*, (14) *Arctia caja*, (15) *Lasiocampa quercus*, (16) *Pachypasa otus*, (17) *Pachymeta flavia*, (18) *Braura truncata*, (19) *Bombyx mori*, (20) *Bombyx meridionalis*, (21) *Bena prasinana*, (22) *Galleria mellonella*, (23) *Clania sp.*, (24) *Chrysopa flava*, (25) *Nephila madagascariensis*, (26) *Nephila senegalensis*.

the spacings of the planes of atoms in the crystal were calculated. It was found that the fibroins could be divided into five groups, the members of each group having the same spacing. Two of these groups could, however, be subdivided on the basis of visual differences in intensity of the two principal equatorial spots. The dimensions of the unit cells for these five main groups are shown in the table. It will be seen that the dimensions in two direction are the same for all the fibroins. These directions are that along the fibre axis (*c*) where

X-ray Classification of the Fibroins

Group	Unit cell dimensions (A)			Typical fibroin
	a	*b*	*c* (fibre axis)	
1	9.3	9.44	6.95	*Bombyx mori*
2 a	10.0	9.44	6.95	*Anaphe moloneyi*
2 b	10.0	9.44	6.95	*Clania* sp.
3 a	10.6	9.44	6.95	*Antheraea mylitta*
3 b	10.6	9.44	6.95	*Dictyoploca japonica*
4	15.0	9.44	6.95	*Thaumetopoea pityocampa*
5	15.7	9.44	6.95	*Nephila senegalensis*

the repeat distance of 6.95 A represents two residues of an almost fully extended peptide chain, and that between chains in the direction of the hydrogen bonds (*b*). The third dimension (*a*) is in the direction of the projecting side chains that distinguish the different amino acids, and this dimension varies for the five groups from 9.3 A to 15.7 A, presumably according to the size of the chain that has to be accommodated.

DISCUSSION

Consideration of the X-ray grouping of the fibroins, their amino acid composition and their biological taxonomy does not reveal any complete system of correlation, but certain observations are possible.

All the fibroins from the *Saturniidae* have high alanine contents, a somewhat smaller amount of glycine, and serine is the third most abundant component. They all belong to the same main X-ray group 3. The *Saturniidae* fibroins, however, can be divided into two sub-groups by X-rays, and the analytical results suggest that this differentiation may also be indicated by the composition since the two fibroins of group 3 b have lower alanine contents

than those af 3 a. It would be interesting to know if there is any corresponding biological taxonomic differentiation.

The fibroin of *Bombyx mori* appears to be an unusual type, characterised by a high glycine content, followed by alanine, serine and tyrosine in decreasing order of abundance, and by X-ray reflections that indicate a close packing of the chains in the crystalline regions facilitated by the small side chains of the glycine residues. This type of fibroin has only been found in our studies in the *Bombyx* genus and in a member of the *Agrotidae*, *Bena prasinana*, that appears remote from *Bombyx* from the standpoint of biological taxonomy.

X-ray group 2 a has so far only been found among the *Thaumetopoeidae* in the *Anaphe* and *Hypsoides* genera, where it is associated with an alanine content of over 50 %. The closely related X-ray group 2 b occurs in a member of the *Psychidae*, *Clania* sp. where it is associated however with a quite different composition. Another member of the *Thaumetopoeidae*, *Thaumetopoea pityocampa*, yields a fibroin very different in its composition and X-ray grouping, and indeed in its physical properties from the *Anaphe* type fibroins. This latter fibroin has, in fact, a close affinity in its composition, X-ray grouping and properties with the fibroins from two other families, *Lasiocampa quercus* and *Arctia caja*.

Of the spider silks examined, those from *Nephila madagascariensis* and from *Nephila senegalensis* are quite different in composition and X-ray grouping. This is almost certainly a reflection of the different functional forms of the fibroins, arising from different glands, the fibroin from *N. senegalensis* being the egg-cocoon fibre and that from *N. madagascariensis* being reeled from the spider, probably the life-line fibre. The other spider silks examined show a much closer affinity with that from *N. senegalensis*.

We have, therefore, in the fibroins a great range of protein filaments which offer enormous scope for further investigation of protein structure and its bearing on the properties of fibres. At present we must regard the fibroin from each species of arthropod as unique in spite of the similarities that exist and the groupings that can be made on the basis of amino acid composition and X-ray crystal structure. « The vast amounts of evolutionary information that may be hidden away » in protein structure (Crick 1958) are still largely obscure in the case of the fibroins, but may yet be revealed when further knowledge of their amino acid sequences is obtained and this may be of assistance in biological taxonomy.

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SUMMARY

We have examined the silk fibroins produced by about seventy species of the *Insecta* and *Arachnida*, covering some 25 families. The qualitative amino acid analysis af all the fibroins and the quantitative analysis of over twenty fibroins have shown that the simple amino acids, glycine, alanine and serine play a major role in their compositions. Examination of the fibroins by X-rays has revealed seven different kinds of structure of the crystalline regions of the fibroins. There are some broad correlations between the amino acid composition, the X-ray group and the biological classification of many of the fibroins but many species are anomalous.

RIASSUNTO

La composizione delle fibroine della seta negli Artropodi.

Abbiamo esaminato le fibroine della seta prodotte da circa 70 specie di Insetti e di Aracnidi, appartenenti a circa 25 famiglie. L'analisi qualitativa degli aminoacidi di tutte le fibroine e l'analisi quantitativa di più di 20 fibroine, hanno mostrato che gli aminoacidi semplici, glicina, alanina e serina hanno una parte preponderante nella loro composizione. Esami delle fibroine per mezzo di raggi X hanno rivelato sette differenti generi di struttura della regione cristallina delle fibroine. Ci sono vaste correlazioni fra la composizione in aminoacidi, il gruppo di raggi X e la classificazione biologica di molte delle fibroine, ma molte specie sono anomale.

DE WILDE J. (*)

ACTION OF THE JUVENILE HORMONE IN THE ADULT COLORADO BEETLE *LEPTINOTARSA DECEMLINEATA* SAY

1. Diapause in the Colorado beetle is primarily a short-day effect. By rearing the beetles under different photoperiods, a predictable percentage of diapause may be obtained in each generation. Studying different photoperiods, a sharp shift occurs at about 15 hrs. Above this value diapause occurs in 100 % of the population. Temperature counteracts the short-day effect only at about 35-40° C. At normal values the temperature plays a minor rôle.

2. Beetles in diapause show several phenomena characteristic of deficiency of the *corpora allata* (pseudo-allatectomy). This was clearly shown in experiments in which *corpora allata* and ovaries were measured in beetles under long- and short-day treatment. In long-day beetles the *corpora allata* are larger and ovarian development generally occurs.

3. In diapausing beetles body weight remains remarkably constant. The rate of respiration (oxygen intake) of such beetles is at about 25-30 % of the normal value.

4. *Effects of allatectomy and re-implantation of corpora allata.* In these experiments, use was made of « long day » beetles only. Allatectomy was performed the day following emergence, using the « neck membrane » technique. To this effect the head was bent downwards by means of a clamp of special design. As a rule the *corpora allata* and *corpora cardiaca* were both removed, but the effect was chequed by reimplanting *corpora allata* only.

a) *Effect on behaviour.*

In both male and female beetles, the effect of allatectomy on behaviour is strikingly similar to that of « short day ». The change of feeding into burrowing responses merely occurs with a somewhat greater delay of time.

Reimplantation of active *corpora allata*, which was performed in 25 female beetles, of which 18 survived for four weeks or more, resulted in re-occurrence of feeding and oviposition in ten cases. The rate of oviposition of these beetles after reimplantation of two *corpora allata* was in some cases the same as with

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normal long-day beetles. Uptill now our attempts to obtain a similar reactivation by injection with *Hyalophora cecropia* extracts have failed.

In the experiments mentioned above the whole post-cerebral complex was exstirpated. We have subsequently made experiments in which only the *corpora allata* were taken out. The results were essentially the same.

b) *Effect on metabolism.*

Oxygen consumption in the operated beetles is on a still lower level than in « natural » diapause. Reimplantation of 4-6 *corpora allata* restores the normal level of respiration (de Wilde and Stegwee, 1958).

5. *In vitro effect of the juvenile hormone.* Experiments with tissue homogenates of active and diapausing beetles showed the same differences in oxygen consumption as are observed with intact insects.

Addition of four active *corpora allata* per « beetle equivalent » to these homogenates to our surprise resulted in a remarkable stimulation of cellular metabolism. An increase of 12-140 % was observed in four series of experiments (de Wilde and Stegwee, 1958).

TABLE I.

Effect of abdominal extract of male adult *Hyalophora cecropia* on oxigen consumption of tissue homogenates of *Leptinotarsa decemlineata* adults (Warburg method).

Added extract mm^3/g	Oxygen consumption $mm^3/g/h$	Oxygen consumption % of blank	(Treatment-blank) P
A. *diapausing 18 h.* ♀			
—	129.0		
30	160.2	124.2	0.99
60	181.9	140.9	0.95
B. *diapausing 10 h.* ♀			
—	57.6		
20	76.5	132.8	0.95
40	90.0	156.2	0.99
C. *Allatectomy* ♀			
—	47.1		
15	60.6	128.6	0.95
30	64.8	137.6	0.99
D. *Allatectomy* ♂			
—	20.4		
15	42.9	210.3	0.99
30	64.8	317.6	0.99

We can now add to these observations the results of our experiments with extracts of abdomens of male adult *H. cecropia.* These extracts made by means of the ether-methanol technique described by Williams (1956) have, according to various authors, in many respects the properties of the juvenile hormone. Injection of 2 mm^3 of the crude extract into allatectomized female beetles resulted in an increase in O_2-uptake varying from 15-875 % lasting for 3-11 days.

Also the effect of this substance on tissue homogenates (Table I) bears resemblance to that of the *corpora allata.* We are now using this in vitro test for juvenile hormone activity in an attempt at concentration and purification of this substance.

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SUMMARY

Diapause in the Colorado beetle is primarily a short-day effect. By rearing the beetles under different photoperiods, a predictable percentage of diapause may be obtained in each generation.

Beetles in diapause show several phenomena characteristic of deficiency of the *corpora allata* (pseudo-allatectomy). This was tested in experiments with extirpation and reimplantation of the *corpora allata* in both sexes. These experiments show that the behaviour changes, the arrest of oogenesis und the low rate of respiration characteristic of diapause are all under the direct control of the *corpora allata.* This humoral effect is on a subcellular level. Oxygen consumption of homogenates of diapausing beetles is significantly stimulated by addition of 2-4 active *corpora allata* per beetle equivalent. Small doses of a juvenile hormone concentrate prepared from the abdomens of male *Cecropia* silkworms have the same effect, in vitro as well as in vivo. This may be a valuable test for assaying the juvenile hormone.

RIASSUNTO

L'azione dell'ormone giovanile nell'adulto del Coleottero Leptinotarsa decemlineata *Say.*

La diapausa nel Coleottero *Leptinotarsa decemlineata* Say. è in primo luogo il risultato dell'abbreviamento del giorno. Allevando Coleotteri in diversi periodi di illuminazione si può ottenere in ogni generazione una prevedibile percentuale di diapausa. Coleotteri in diapausa mostrano parecchi fenomeni caratteristici inerenti alla minore efficienza dei *corpora allata* (pseudo-allatectomia). Questo fu provato, in alcuni esperimenti, con l'estirpazione e il ritrapianto dei *corpora allata* in ambo i sessi. Questi esperimenti mostrano che cambia il comportamento, che l'arresto della oogenesi e il basso tasso respiratorio caratteristici della diapausa sono tutti sotto il diretto controllo dei *corpora allata.* Questo effetto umorale è su di un livello sottocellulare. Il consumo di ossigeno di omogenati di Coleotteri in diapausa è stimolato in modo significativo dall'aggiunta di 2-4 *corpora allata* attivi per equivalente Coleottero. Piccole dosi di concentrato di ormone giovanile, preparato dagli addomi dei maschi dei bachi da seta *Cecropia*, hanno lo stesso effetto sia *in vitro* che *in vivo.* Questo può essere un test valevole per il saggio dell'ormone giovanile.

STEGWEE D. (*)

METABOLIC EFFECT OF A CORPUS ALLATUM HORMONE IN DIAPAUSING *LEPTINOTARSA DECEMLINEATA* SAY

In the paper given at this Symposium by De Wilde (1960), mention was made of experiments which showed that succinate oxidation by tissue homogenates of diapausing adult Colorado potato beetles was enhanced by the addition of a factor, which might be a or the corpus allatum hormone. This was inferred from the fact that stimulation of succinate oxidation could be obtained as well by the addition of corpora allata from active Colorado potato beetles as by the addition of an ether extract from abdomens of male *Hyalophora cecropia* moths, which extract was known to contain considerable amonts of corpus allatum hormone (De Wilde, Stegwee, 1958; De Wilde, Bink, 1959).

Since, the effect of cecropia-extract upon tissue respiration in the diapausing Colorado potato beetle was studied more in detail by the present author in close collaboration with Mrs. A. R. van Kammen from the Laboratory for Comparative Physiology, University of Amsterdam.

Using the spectrophotometric methods of Cooperstein et al. (1950, 1951) the activities of « succinic dehydrogenase » and cytochrome oxidase were determined separately in crude homogenates of whole beetles. As was to be expected, in diapausing animals the activities were lowered to a considerable extent (by 70-80 %) as compared with active insects. No effect of cecropia-extract upon the activities of the enzymes in homogenates of active beetles was observed. A different situation, however, was encountered when working with diapausing animals. Within a limited concentration range the cecropia-extract exerted a distinct stmulating effect upon the « succinic dehydrogenase » system, whereas the cytochrome oxidase remained unaffected (Fig. 1). So it seems that the site of stimulation of the succinoxidase system lies somewhere in the part of the respiratory chain between succinate and cytochrome c.

It was thought that suspensions of isolated thoracic mitochondria (sarcosomes) might provide a more suitable system for further studies. Such suspensions could easily be prepared from thorax homogenates by fractionated centrifugation in a sucrose-versene medium.

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Sarcosomal preparations from active beetles readily oxidized succinate and α-glycerophosphate ($QO_2 = 30 - 40$), although it should be stated that the oxidation of the latter substrate proceeded at a relatively slow rate as compared with the rates found in the House-fly (Chance, Sacktor, 1958) and the American

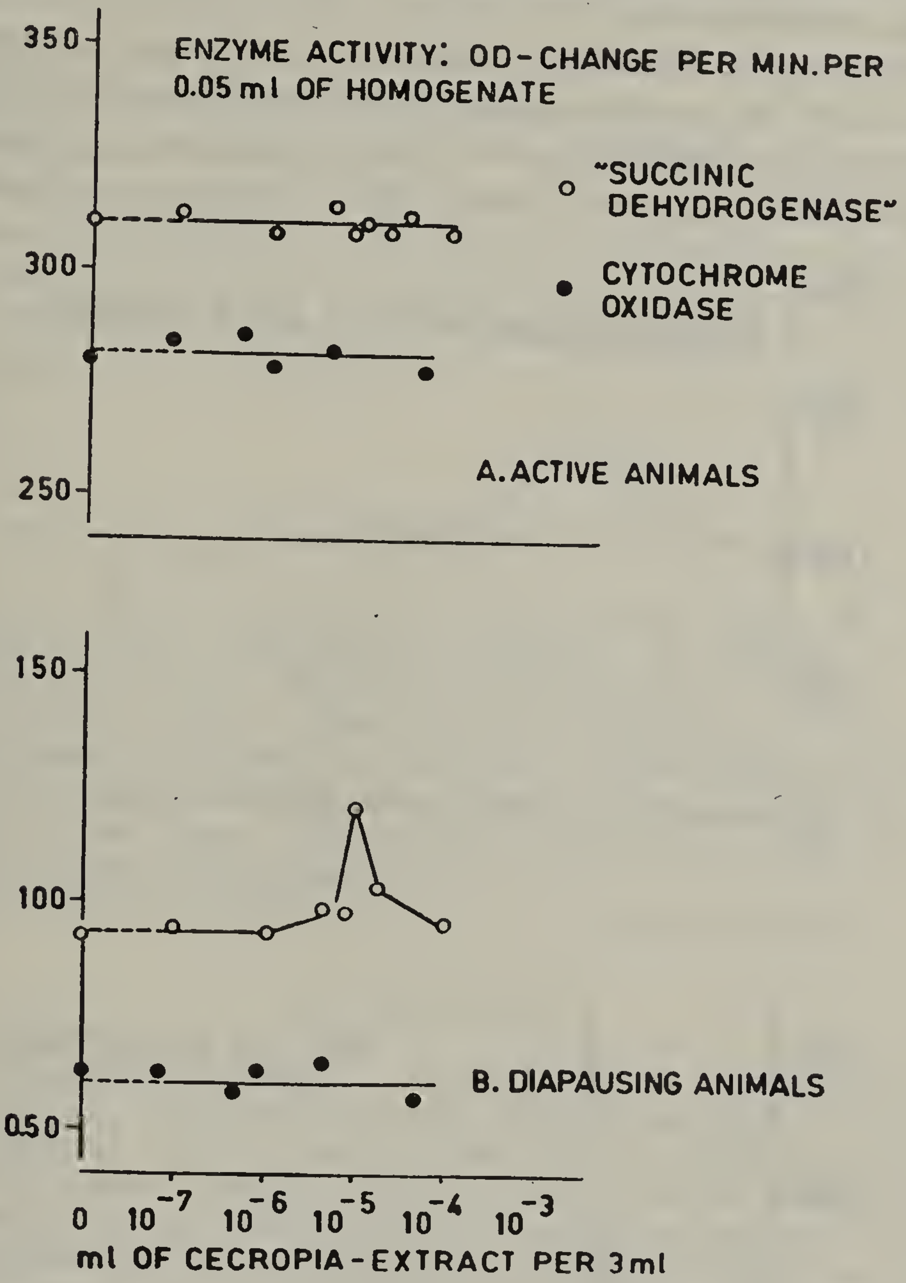

Fig. 1
Effect of Cecropia-extract upon the succinoxidase system in crude homogenates of adult Colorado potato beetles.

roach (Cochran, King, 1960). The preparations also showed an appreciable phosphorylative activity when tested in a suitable medium, the P:O ratio being about 0.6. No effect of cecropia-extract upon either respiration or phosphorylation was detected.

Diapausing beetles yielded sarcosomal preparations with a very low respiratory activity (QO_2 for succinate 3-4). The P:O found with these preparations was also about 0.6. Cecropia-extract was able to stimulate the oxidation of suc-

cinate by diapause-sarcosomes, although this was rather inconsistent. The oxidative phosphorylation was stimulated in a far more pronounced way. Again, this stimulation was found within a narrow range of rather low concentrations of cecropia-extract. At higher concentrations the extract appeared to uncouple oxidation and phosphorylation (Fig. 2).

As for its effect upon oxidative phosphorylation the cecropia-extract shows a resemblance to the thyroid hormone thyroxine. This hormone was also reported to stimulate phosphorylation at low concentrations and to uncouple it at higher ones (Dallam, Howard, 1960). The cecropia-extract seems different,

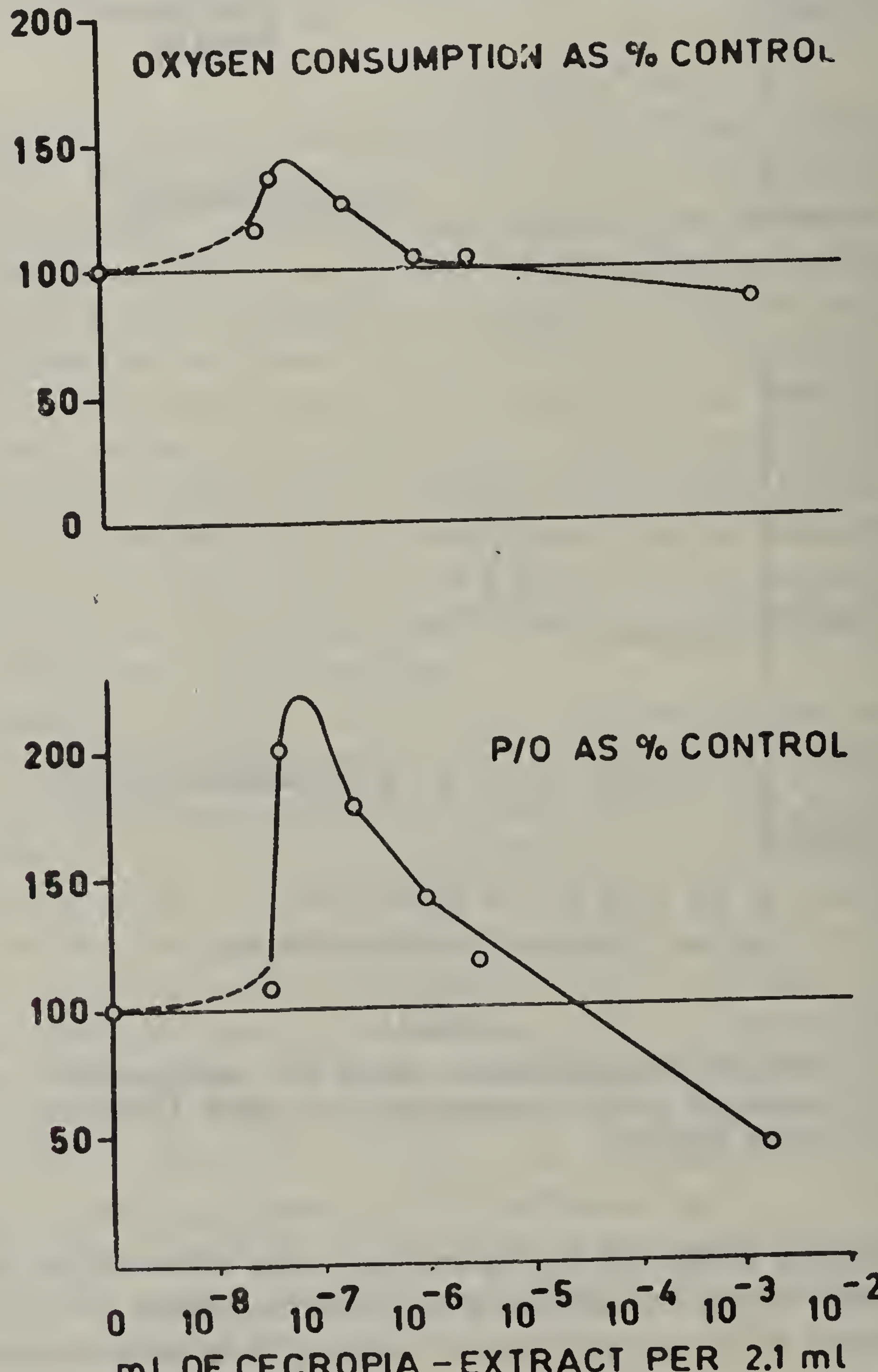

Fig. 2

Effect of Cecropia-extract upon respiration and phosphorylation of sarcosomes from diapausing adult Colorado potato beetles.

however, with respect to its effect upon oxidation, but this effect still is somewhat uncertain. In this respect it is worth while to briefly mention the following observations.

In the course of our attempts to isolate sarcosomes from active beetles it appeared that the supernatant obtained after sedimentation of the sarcosomes contained a substance which considerably stimulated respiration and oxidative phosphorylation by the sarcosomes with succinate as substrate. Independently, a similar phenomenon was observed by Van den Bergh, Slater (1960) in Houseflies. These observations point to the fact that obviously in the commonly used insect sarcosomal preparation the conditions for oxidation and phosphorylation are far below the optimum. Experiments are in progress now to study these processes under more favourable conditions and it is hoped that they will help to elucidate the mechanism of action of the corpus allatum hormone upon metabolism.

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SUMMARY

A substance — assumedly a hormone — derived from the *corpora allata* of active Colorado potato beetles exerts a stimulating effect upon tissue respiration of diapausing beetles, using succinate as substrate. This respiration was also stimulated by an ether extract of abdomens of male *Hyalophora cecropia* moths, which contains considerable quantities of *corpus allatum* hormone. In phosphorylating sarcosomal preparations obtained from the thoracic muscles of diapausing beetles the oxidative phosphorylation was even more stimulated.. Some evidence points to the reaction between succinate and cytochrome c as the site of stimulation.

RIASSUNTO

Effetti del metabolismo dell'ormone del corpo allato nella diapausa di Leptinotarsa decemlineata *Say.*

Una sostanza — presumibilmente un ormone — derivata dai *corpora allata* di adulti attivi del Coleottero *Leptinotarsa decemlineata* Say., esercita un effetto stimolante sulla respirazione del tessuto del Coleottero in diapausa, usando succinato come substrato. Questa respirazione fu anche stimolata da un estratto etereo degli addomi di maschi del Lepidottero *Hyalophora cecropia*, che contiene quantità considerevoli di ormone del *corpus allatum*. In preparazioni sarcosomali fosforilate ottenute dai muscoli toracici di Coleotteri in diapausa la fosforilazione ossidativa fu ancor più stimolata. Qualche fatto mostrerebbe le reazioni fra succinato e citocromo c come luogo di stimolazione.

Hrdy' I., Novák V. J. A. (*)

A CONTRIBUTION TO THE QUESTION OF NON-SPECIFICITY OF THE EXOHORMONES

The existence of substances the presence of which prevents the development of normal sexuals inhibiting first of all the development of ovaries and causing the special changes in the instinctive behavior as known in the worker caste of the social insects, has been shown in termites (Light 1944; Lüscher 1952, 1956 a, b) for the first time and in the honeybees afterwards (Butler 1954 etc.). The interest in this type of substances is notably increased by the recent finding that their effect, depending in inhibition of growth without any similar effect on metabolism and other physiological functions of the insect body, might be a common character of a wide range of organic substances, as e. g. the unsaturated fatty acids (Sláma 1960, c.f. Novák 1960). This is in full agreement with the findings of Butler and Carlisle 1956, on the influence of the queen inhibitory substance upon the development of ovaries in the common prawn *Leander serratus* (Rath), showing the non-specificity of this substance between Insects and Crustaceans. The showing of a similar non-specificity of the queen bee exohormone (1) between the order of *Hymenoptera* and that of *Isoptera*, the other independant group of social insects, has been the subject of the present communication.

The efficiency of extractions prepared from one — and more — years-old egglaiyng queen bees (*Apis mellifica*) was tested on the groups of pseudergats of the termite *Kalotermes flavicollis* (F.). Two series of experiments were performed with groups containing twenty individuals each. Extracts in 96 p. c. alcohol were used evaporated in vacuum and emulgated in destilled water in proportion of 1 ml per 1 queen. For each experimental colony 1 ml extract was put on the filtering paper 30 × 20 mm of sive and after drying put into the chamber of the respective group as the only food. Control groups obtained the

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(1) The authors prefer the term exohormones for this type of substances to that of pheromones recently suggested by Karlson and Lüscher out of the reason given by Novák (1960, p. 270).

same filter paper without extract. The number of supplementary reproductives developed and their mortality were ascertained in 2-6 days intervals during one month following the administration of the extract.

In control groups the larvae developing to supplementary reproductives could be found as early as after 4-8 days (registered according to appearance of the pigmented imaginal eye-discs). After 12-14 days when the superfluous supplementary reproductives were eliminated their number was stabilized to

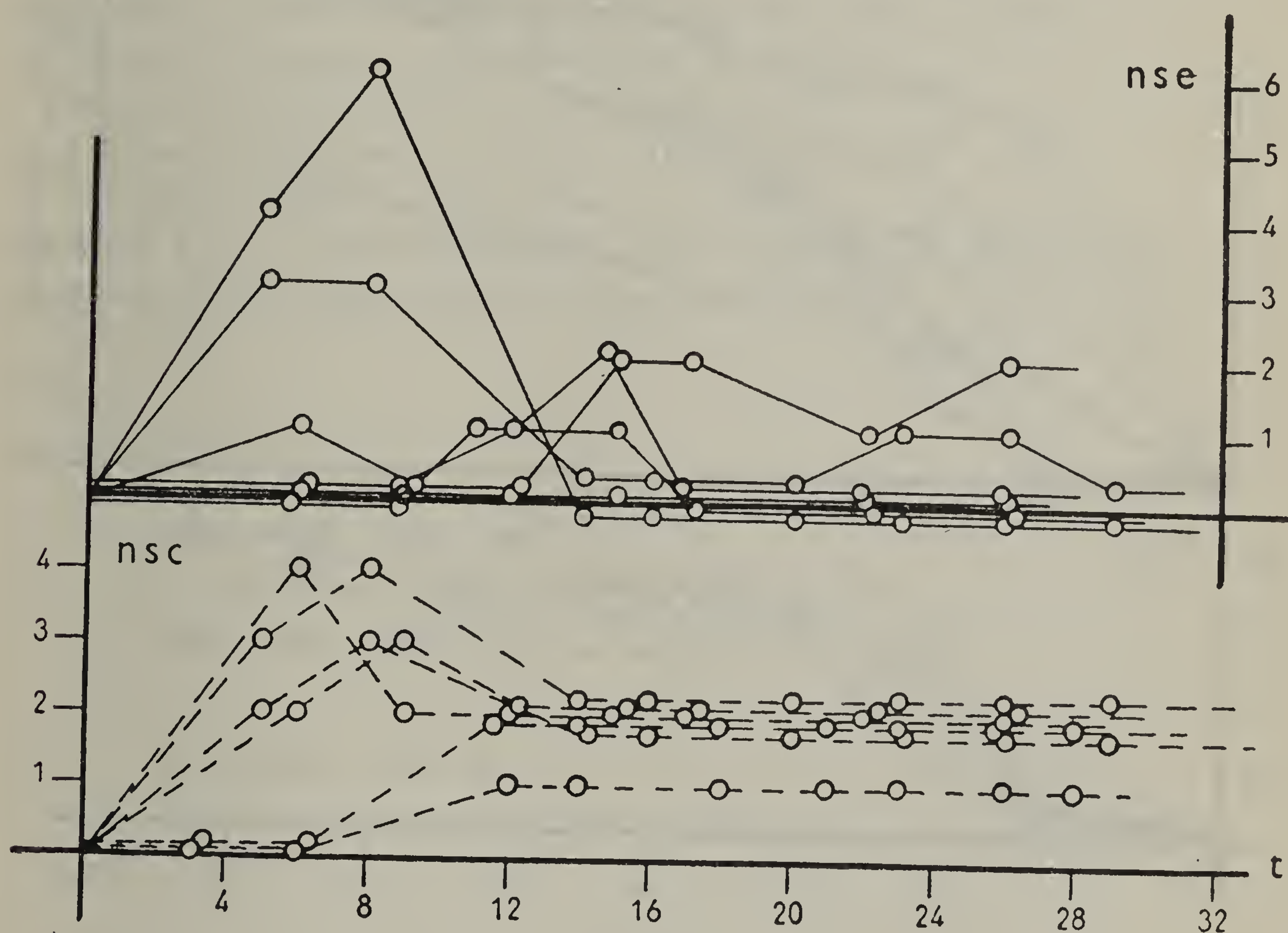

Fig. 1

Appearance of supplementary reproductives in groups of Kalotermes flavicollis pseudergates. n-se: number of supplementary reproductives in colonies with extract; nsc: in control groups; t: time in days.

one pair (except one colony where one female only developed). On the other hand, in groups fed with the queen extract the larvae developing to supplementary reproductives were found only in a part of the colonies with considerable retardation, in all the colonies except one the supplementary reproductives having perished before the experiments were finished (see Fig. 1).

The total mortality in the moment of termination of experiments did not excede 40 p. c. in controls, whereas in colonies with exohormones it varied bet-

ween 40-80 p. c. This increased death-rate is at least partly due to the above-mentioned activ elimination of the supplementary reproductives by workers (see Fig. 2).

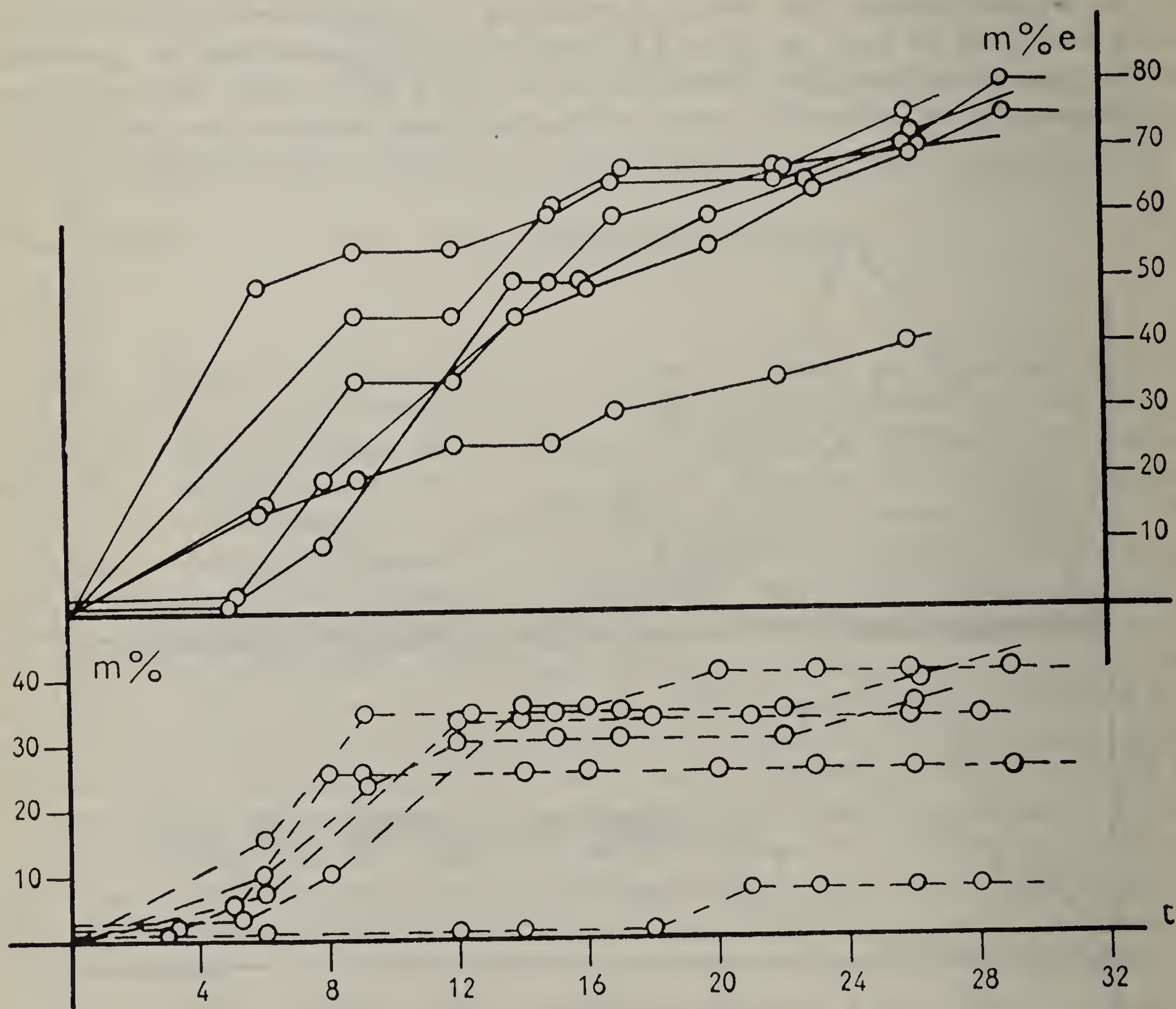

Fig. 2.

Mortality during experiments. m % e: per cent mortality in colonies with extract; m %: in control groups; t: time in days.

Although these results demand further evidence on a larger material they seem to confirm the expectation, that to the non-specificity of effect between the class of insects and that of crustaceans (Butler and Carlisle 1956) corresponds the non-specificity within the narrower scope of the class of insects. From this point of a view the studied queen bee exohormone seems to correspond to the neurohormones of both crustaceans and insects (as e. g. the activation hormone) and to the insect moulting hormone (ecdyson) the unspecificity of which was proved by Karlson (1957) recently.

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JUCCI C. (*)

BIOCHEMICAL ASPECTS OF THE SYMBIOTIC RELATIONSHIP BETWEEN COCKROACHES AND MICRORGANISMS IN THE BACTERIOCYTES OF THEIR FAT-BODY

Results achieved in the last few years by several research workers in different countries — chiefly however by Brooks and Richards in USA — offer at last a partial solution to the problems that have chiefly interested me, since 1924 when I discovered in *Mastotermes darwiniensis* bacteriocytes of fat-tissue and interpreted them as indication of a symbiosis transmitted from Protoblattoids to two divergent lines of descent, *Blattoidea* and *Isoptera.*

In our « Spallanzani » Institute in Pavia research work has now been resumed on several species of roaches: *Blattella germanica, Blatta orientalis, Periplaneta americana, Nauphoeta cinerea*: with antibiotics given *per os* we are trying to obtain aposymbiotic strains like those Brooks and Richard obtained with aureomycin.

On other species of cockroaches, like *Blaberus craniifer*, we are trying parenteral administration (injecting in the blood) to attempt to obtain the destruction of the symbionts in treated individuals, and not only the lack of symbionts in their progeny (Jucci, Fava, Laudani).

At the same time Manunta together with Bernardini progresses in her chromatographic researches and, with younger research workers, begins to essay, by Warburg's methods, the respiratory activities of bacteriocytes in normal conditions and in the course of intoxication with insecticides interacting with neurotropic drugs.

Having worked out a method for the dissociation and separation — by differential centrifugation — of the bacteriocytes of *Blaberus craniifer* from the fat-body, Manunta has investigated, by means of paper chromatography, a series of vitamins, such as PP, B_1, B_2, folic acid an panthotenic acid; and for this last substance has obtained a positive result: higher concentration of the vitamin in the isolated bacteriocytes. Manunta stresses the point that panthotenic acid is the basic component of a very complex coenzime, COA, which is of great

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importance for the activation of some substrate in the biological acetilation and in synthesis and break-down of fat substances. She has put forward the hypothesis that one of the functions of the symbiont, in cockroaches fat-body, is just the synthesis of this vitamin, by condensation of β-alanine with α-dioxi-ββ-dimetilbutirric acid.

We intend to persist, as we did unsuccessfully many years ago, in two main directions: 1) in the vitro culture of bacteriocytes, now more hopeful since the very difficult problem of tissue culture in insects has made some substantial progress; 2) the transplantation of bacteriocytes into the body of insects like silkworms and termites.

Since it is possible (according to the fine research of Brooks and Richard) to mantain aposymbiotic cockroaches by giving them vitamins and aminoacids — presumably provided under ordinary conditions, by symbionts — with suitable dietetic essays on aposymbiotic roaches (filled up again with symbionts through the implantation of fat-body) it is possible to analyse the problem of symbiotic equilibrium. The comparison between normal insects and those reared on a vitamin-free diet or even with eccess of these vitamin factors could produce useful results.

What is essential is to shake up, in some way, the symbiotic equilibrium and try to break up the harmonic relationship obtaining, for instance, lysis of bacteria or phagocitosis or, on the other hand, a septicemic invasion of the host-organism.

We are beginning also to investigate the influence, on the normal symbiosis, of natural parasitization with sporozoa, and we intend to essay artificial parasitization with yeast or fungi.

We intend to insist on two chief directions in order to shake up the symbiotic equilibrium and so open the way to a deeper analysis of the simbiotic relationship:

1) modification of the genetic constitution of the microrganism;

2) modification of the genetic constitution of the host.

Operating on several species of roaches and with various antibiotics, we want to effect a partial sterilization of the symbiotic flora. From the survivors it should be possible to constitute strains resistant to antibiotics (penicillin, streptomycin, sulfamido - resistant) so after a period of inibition, they could fill up again the empty bacteriocytes. These resistant microrganisms could present physiological activities mutated also in other ways, and the exploration of a new behavior in symbiotic relationship should make a more profound analysis of the phenomena possible.

The other possibility we are considering is the introduction of mutation in symbiotic bacteria by radiation (with X-rays or with other ionizing radiation) of the female cockroach (or of her embryos) or physical or chemical treatment of bacteriocytes: separated, as for chromatographic essay, or still included in the fattissue to be transplanted in aposymbiotic individuals.

Disrupting — through mutation induced in the microrganism or even in the host — the genetic coaptation between the insect and the symbiont, we can hope to get a deeper understanding of the biochemical basis of the symbiotic relationship.

A similar kind of research, on different species, could be instructive on the evolution of these symbiotic relationship since different species, while similar in the general outline of the phenomena, differ in details, such as: 1) number and disposition of bacteriocytes in fat-tissue tubes; 2) size of symbiont and their fine structure; 3) transmission of symbionts and their behaviour during embryonic development.

SUMMARY

A brief account is given of the researches undertaken and of the experiments planned on several species of *Blattoidea*, in order to investigate, comparatively, the basis of the symbiotic relationship.

RIASSUNTO

Aspetti biochimici delle relazioni simbiotiche fra Blattoidei e microrganismi nei batteriociti del corpo grasso.

Breve riassunto delle ricerche intraprese e dei programmi di lavoro su diverse specie di *Blattoidea* per lo studio comparativo delle basi delle relazioni simbiontiche.

STEGWEE D. (*)

SOME OBSERVATIONS ON THE MODE OF ACTION OF TETRAETHYLPYROPHOSPHATE IN THE HOUSE-FLY

This paper will deal with the possible physiological significance of inhibition of the enzymes acetylcholinesterase (AChE) and ali-esterase (AliE) by tetraethylpyrophosphate (TEPP) in the house-fly.

To avoid confusion the enzymes are defined as follow. AChE is the enzyme which hydrolyses acetylcholine (ACh) following kinetics which give rise to the well-known bell-shaped pS-curve. The enzyme is completely inhibited in vitro by eserine at 10^{-5} M. AliE hydrolyses a variety of esters such as methyl and ethyl butyrate,, phenylacetate and triacetin, but not ACh. The enzyme is not inhibited in vitro by eserine up to 10^{-4} M, but can be completely inhibited by TEPP at 10^{-6} M.

It has been shown (Stegwee, 1959) that in house-flies treated with TEPP prostration coincided with inhibition of both AChE and AliE, the inhibition percentages being 50 and 95 respectively. In spite of the high degree of AliE inhibition, it remained doubtful whether this was of any direct physiological or toxicological importance. For, when tri-*o*-cresyl phosphate (TOCP) was applied to house-flies a similar high degree of in vivo AliE inhibition developed, however without any inhibition of AChE and also without the occurence of any symptoms of poisoning. It was, therefore, concluded that the occurrence of these symptoms was most likely due to AChE inhibition.

As was already emphasized by Smallman and Fisher (1956) the hypothesis that organophosphorus insecticides act by inhibiting AChE requires that ACh is accumulated during poisoning. This was actually found to be so by the above authors in the case of different organophosphorus compounds, among which TEPP. This could be confirmed by the present author (Stegwee, 1960).

Now two questions arise. The first is: how is accumulation of ACh feasible with only 50 per cent of the AChE inhibited? An answer to this question is not readily given. However, it should be borne in mind that the inhibition values reported all refer to over-all inhibition. This means that there is a possibility

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that at certain critical sites in the nervous system the AChE inhibition reaches much higher values. If it is true that AChE exerts its function only at the synapses, then it follows, e.g., from the work by Colhoun (1959) that the insects' nervous system contains considerable amounts of « non-functional » AChE. Also, as was stated by dr. Howden in a discussion in the physiology section of this Congress, in the head of the house-fly there seem to occur two types of AChE, hitherto not distinguished. These two types would have a different spatial distribution in the brain and possibly they are functionally different too. All this may indicate that the significance of the 50 per cent over-all inhibition of AChE should not be over-estimated.

The second question is: is the ACh which accumulates after TEPP poisoning actually « free » ACh, as is to be expected from theoretical considerations? Experiments, designed to give an answer to this question were carried out in our laboratory by Mr. Voorma. It was found that in the house-fly after a dose of 0.4/μgm of TEPP, the total ACh content of the head increased sharply within the first hour after application and less pronounced during the next two to three hours. Values of 250 per cent of the controls were found. Determinations of free ACh demonstrated that the increase in total ACh was equal to the increase in free ACh, the bound fraction remaining virtually constant. This would seem a very good confirmation of the theory, were it not that in untreated flies invariably also a considerable amount of « free » ACh was found. This amount depended largely on the way of preparation of the extracts, but even in extracts which were prepared with the utmost care at least about 40 per cent of the total ACh occurred in the free form. This agrees findings by Pal and Smallman (1956) and Colhoun (1958). Unfortunately, however, the consequence must be that at present and as far as the house-fly is concerned, a discussion about « free » and « bound » ACh is bound to be of little value in relation to the mode of action of insecticides. Nevertheless, we feel that our experiments carried out thus far are not too disappointing and that better techniques possibly will prove the clue to the ultimate solution of the underlying problems.

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SUMMARY

Evidence is presented that tetraethylpyrophosphate acts primarily on the enzyme acetylcholinesterase. The resulting accumulation of acetylcholine should be held responsible for the observed symptoms of poisoning. In agreement herewith it could be shown that the accumulated acetylcholine occurred in the « free » form. The significance of this finding is discussed, especially in view of the fact that — contrary to expectations — in untreated insects also considerable amounts of « free » acetylcholine are found.

RIASSUNTO

Alcune osservazioni sul modo di azione del tetraetilpirofosfato nella Mosca domestica.

Si mette in evidenza che il tetraetilpirofosfato agisce soprattutto sull'enzima acetilcolinesterasi. L'accumulazione risultante di acetilcolina potrebbe essere considerata responsabile di sintomi osservati di avvelenamento. In accordo con ciò si potrebbe ritenere che l'acetilcolina accumulata era presente nella forma « libera ». Il significato di questa scoperta è discusso, specialmente in vista del fatto che — contrariamente alle aspettazioni — in Insetti non trattati è anche presente una quantità considerevole di acetilcolina « libera ».

COLHOUN E. H. (*)

BLOOD FACTORS IN INSECTICIDAL POISONING

INTRODUCTION

Sternburg et al (1957) have suggested that after treatment of *Periplaneta americana* L. with DDT, that a blood toxin accumulates in the blood with eventual disruption of the entire nervous system. Should this supposition be correct then it should be possible to test the importance of blood substances as a primary lesion in poisoning by the technique of parabiosis (Bodenstein, 1953). It would appear that an experiment of this kind is of some importance for Spiller (1955) showed that *Rhodnius* prostrated by a massive dose of DDT did not kill *Rhodnius* to which they were joined in parabiosis. The results reported briefly below form part of a program dealing with pharmacological effects of chlorinated hydrocarbon poisoning in *Periplaneta*, (Colhoun and Spencer, in preparation).

Cockroaches treated topically with a lethal dose of either DDT, dieldrin or TEPP were joined to untreated roaches. The intoxicants used were of greatly different molecular structure but induced common symptoms of hyperactivity and convulsions followed by prostration in the treated roaches. Cyanide-treated roaches were used to study the effect of a dying roach upon another to which it was joined in parabiosis.

RESULTS

When just prostrate roaches treated topically with either DDT, dieldrin or TEPP were joined to normal healthy roaches. A circular piece of cuticle was removed from the pronotal shield of a pair of roaches and after placing the roaches back to back the pronotums were cemented together in a manner similar to that devised by Bodenstein (1953). The results of these experiments given in Table 1 show that union of untreated roaches with other treated with the insecticides brought about death of the untreated roaches. These results are contrary to those reported by Spiller (1955) for *Rhodnius* treated with DDT.

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However it is apparent that regardless of the insecticide used that the normal roaches died within the same time factor and that no ill-effects were apparent for the first 48 hours after parabiosis. These facts are of importance in deciding the cause of death in the untreated roaches.

The possibilty existed that a dying roach could cause a normal roach to die when they were joined together. Accordingly roaches killed by exposure to potassium cyanide vapour and joined to normal roaches. The electrical nervous activity of a number of the treated roaches was found to be blocked. It is evident from Table 1 that the untreated roaches died and significantly within the same time factor found for the other treatments. The cyanide-treated roaches showed greater necrosis than those treated with either DDT, dieldrin or TEPP when the normal roaches died. Necrosis was observed as a darkening of the cuticle and autolysis of internal organs.

The external symptoms of chlorinated hydrocarbon and organophosphorus poisoning are similar with respect to a period of unrest and hyperctivity followed a phase of convulsive tremors terminating in prostration. Although prostrate, roaches continued to exhibit tremors for some time afterwards. These symptoms were not observed in joined dying untreated roaches. For 48 hours after parabiosis was begun the untreated roaches appeared normal. The treated roaches were no longer showing tremors at this time. Continued observation of the untreated roaches showed the appearance of lethargy, an inability to maintain normal posture and eventual prostration. Infrequent leg tremors were noted in the early stage of prostration but not of the same kind seen in prostrate insecticide-treated roaches. Electrical nervous activity was measured in different regions of the nervous system of prostrate roaches joined to roaches treated with DDT. No evidence was obtained of volleys of nerve impulses easily discernible in roaches treated with DDT. This information led to the supposition that insecticides were not being translocated to the untreated roaches and chemical analysis of these roaches failed to detect the presence of the intoxicants although they were easily recovered from the treated insects.

One of a pair of normal roaches joined in parabiosis was treated with dose of either DDT, dieldrin or TEPP. The dose used was in excess of that amount causing intoxication in a singly-treated roach. The results of these experiments given in Table 2 show that the untreated roaches rapidly developed symptoms of poisoning characteristic of the treatment used. The time of appearance of symptoms and that to knockdown was strikingly similar to treatment of individual insects with the poisons. Chemical analysis of the untreated prostrate roaches showed the presence of the insecticides applied topically to the treated roaches. Furthermore the rapid translocation of the poisons from the treated to the untreated roaches dispelled doubt of the inability of a highly intoxicating agent requiring considerable time to make its appearance in the untreated roach. It is of interest to point out that Beament (1958) showed that a paralysis factor induced in *Periplaneta* pinned to a block was transmitted to a normal roach within 24 hours of commencing parabiosis.

TABLE 1.

The effect of roaches treated with either DDT, dieldrin, TEPP or cyanide upon untreated roaches joined in parabiosis.

Treatment	Number Treated	Stage of treated Roaches at parabiosis	Time in hours and numbers of prostrate untreated roaches after parabiosis									Symptoms in untreated roaches	Insecticides in untreated roaches
			12	24	36	48	72	96	120	144	360		
Controls	15	—	0	0	0	0	0	0	0	0	0	—	—
DDT. 50 ug per roach	11	hyperactive and just prostrate	0	1	0	3	5	1	0	1	—	lethargic before prostration. No tremors as seen in the treated roaches	none
dieldrin 10 ug per roach	7	convulsive to just prostrate	0	0	0	1	4	2	—	—	—	As in DDT	none
TEPP. 5 ug per roach	3	just prostrate	0	0	0	0	1	0	0	2	—	As in DDT	No inhibition of AChE
	3	prostrate 7 hours	0	0	0	0	1	2	—	—	—		
	3	prostrate 9 hours	0	0	0	0	2	0	1	—	—		
30 min. exposure to KCN vapour	6	completely quiescent electrical activity of nervous system blocked	0	0	0	1	2	1	1	1	—	similar to other treatments	not examined

DISCUSSION

When compared with the results of Spiler (1955) for *Rhodnius* it is evident that *Periplaneta* joined in parabiosis after treatment with DDT is unable to survive. A like result was found after treatment with either dieldrin, TEPP or cyanide. For DDT-treatment a distinct difference has been found between two insect species. Is this difference due to the production of an intoxicating agent in the blood of *Periplaneta* treated with the insecticides? The fact that a number of chemically unrelated compounds produced a similar result in roaches joined in parabiosis must be considered when answering this question. Should these poisons produce identical effects in the treated roaches then we are confronted with a universal mode of action in one insect species. On the other hand it possible that a normal cockroach is unable to withstand the metabolic effects of a dying cockroach when joined to it in parabiosis regardless of the treatment used to cause death in the treated insect. This explanation, if valid, would show a fundamental difference in the biochemistry and physiology of *Periplaneta* and *Rhodnius*. A consideration of the time to cause symptoms in the untreated roaches and lack of evidence of symptoms indicating insecticidal-like action, casts doubts upon the role of blood substances as being primary agents in intoxication. The suggestion of Sternburg et al (1937) that DDT-treatment produces a toxin which may disrupt the functioning of the nervous

TABLE 2.

Treatment of one of normal roaches joined in parabiosis with either DDT or TEPP.

Treatment	Appearance of symptoms in treated roaches (hours)	Time to knockdown in untreated roaches	Symptoms in untreated and treated roaches	Insecticide action in untreated roaches
DDT. 50 ug per roach	10 - 18	none at 48 hours	none in untreated roach	none
DDT. 100 ug per roach	12 - 20	18 - 36 hours	hyperactivity prostration	electrical nervous activity abnormal
TEPP. 5 ug per roach	immediate	none at 24 hours	none in untreated roach	——
TEPP. 10 ug per roach	immediate	17 - 40 mins	hyperactivity prostration	100 per cent inhibition of AChE. Electrical nervous activity abnormal

Controls. Treatment of individual roaches with TEPP or DDT.
DDT. 50 ug per roach. Prostrate in 16 - 20 hours.
TEPP. 5 ug injected per roach. Prostrate 1 - 3 mins.

system is difficult to reconcile with the present results if the toxin is the causal agent in promoting external symptoms of hyperactivity and convulsions.

There can be no doubt pharmacologically active substances have been found in the blood of roaches treated with a number of insecticides. (*DDT*: Sternburg and Kearns, 1952; Colhoun, 1959 a. *Dieldrin*: Colhoun, 1959 b. *TEPP*: Colhoun 1958 and 1959 b). Sofar evidence has not been obtained that the blood of a single roach would contain sufficient of these active substances to cause autointoxication. For example when investigating the role of the acetylcholine system in TEPP poisoning in *Periplaneta*, Colhoun (1958 and 1959 a) found acetylcholine in blood following the convulsive phase before prostration. The amount of acetylcholine in blood was only a fraction of that necessary to cause intoxication when acetylcholine was injected into roaches (Colhoun and Spencer 1959 c). The inability of high titers of acetylcholine to intoxicate roaches appears to be non-penetration into the nervous system and the absence of the acetylcholine mechanism at neuromuscular junctions.

The determination of acetylcholine in the blood of roaches treated with an organophosphorus compound is evidence of interference with the cholinergic system within the cockroach. Likewise the toxin of Sternburg et al (1957) may be of some importance in determining a lesion in chlorinated hydrocarbon poisoning, particularly as no evidence has yet been obtained that the toxin is a naturally occuring chemical in tissues of *Periplaneta*.

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SUMMARY

American cockroaches (*Periplaneta americana*) treated with a number of chemically unrelated insecticides were joined in parabiosis to untreated cockroaches. Within a few days the untreated roaches died without exhibiting symptoms of insecticidal intoxication. These results are discussed in relation to blood factors being causal agents in chlorinated hydrocarbon and organophosphorus intoxication.

RIASSUNTO

Fattori dell'emolinfa nell'avvelenamento per mezzo di insetticidi.

Individui di *Periplaneta americana* trattati con un certo numero di insetticidi chimici senza alcun rapporto fra loro, furono uniti in parabiosi a individui non trattati. Nel giro di pochi giorni gli Insetti non trattati morirono senza presentare sintomi di intossicazione da insetticida. Questi risultati sono discussi in relazione ai fattori dell'emolinfa essendo questi agenti causali nella intossicazione con idrocarburi clorurati e con prodotti fosforati organici.

SANBORN R. C., HASKELL J. A. (*)

CHEMICAL REQUIREMENTS FOR THE GROWTH OF INSECT TISSUES *IN VITRO* (1)

Haskell (1959) reported that the basal culture medium of Wyatt (1956) serves adequately for the growth of various tissues of larval Lepidoptera *in vitro* but requires the addition of certain supplements for proliferation of explants derived from pupae. For instance, the migration and mitosis of ovarian cells of diapausing pupal *Samia cynthia* (Cynthia) requires supplementing the medium with aqueous adrenal cortical extract of beef (2). Grace (1958) has reported that the addition of vitamins of the B-complex at concentrations of 0.01 micrograms per milliliter to ovarian cultures of *Bombyx mori* prolongs the survival of isolated cells in the absence of adrenal cortical extract. Since it is possible that the aqueous extract of beef adrenal cortex owes its ability to stimulate migration and mitosis to its content of B-vitamins we have investigated the stimulation of cultures by individual members of this group.

MATERIALS AND METHODS

Pupae of *Samia cynthia* (Cynthia) were purchased from collectors in the New York City area. These pupae were usually collected in late fall or early winter and after their arrival at our laboratory were stored at 5° C. Following surface sterilization pupal ovaries were removed through a dorsal abdominal incision and placed in a small Petri dish containing culture medium; they were then cleared of adhering fat-body without damaging the oviducts; finally, ovaries were bisected and two cultures made from each ovary.

The medium of Wyatt (1956) without the addition of *Bombyx mori* hemolymph was sterilized by passage through an ultra-fine sintered glass filter and

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(2) Lot No. LE 963, obtained through the courtesy of Dr. R. Stafford, The Upjohn Company, Kalamazoo, Michigan.

10 micrograms per milliliter each of penicillin, streptomycin and nystatin were added. To this medium was added 10 % of phenylthiourea-saturated pupal Cynthia hemolymph.

Cultures were prepared as hanging or sitting drops in a culture chamber consisting of a 16 × 22 mm hemocytometer cover glass waxed to an appropriately sized brass ring. One-tenth ml of culture medium was pipetted as a rounded drop into the center of each cover glass and a hemi-ovary was transferred to this drop. After sealing the brass ring and attached cover glass to a microscope slide, the cultures were incubated at 28° C.

The effectiveness of each of the treatments was adjudged by removing the explant from the cover glass, mixing the medium with a fine needle to disperse the cells, inverting the cover glass over a standard blood counting chamber, and counting healthy cells at 200 × magnification. The cells wich are counted by this technique are those which have migrated from the explant or which have arisen by mitosis in the medium outside the original explant.

RESULTS

Confirming the observations of Grace (1958), we find that the addition of choline, biotin, *m*-inositol, folic acid, riboflavin, niacinamide, thiamine, para-aminobenzoic acid, pyridoxine, and calcium pantothenate to the culture medium at a level of 0.01 micrograms per ml increases the mean number of free cells by approximately forty-fold (Table 1). Although it cannot be quantitatively measured, addition of all vitamins also prolongs the survival of cells in tissue culture.

The effects of the individual vitamins may be determined in either of two ways: We might arrange a series of cultures, adding to one group a single vitamin; to another group, a second of the vitamins and so on until the effect of each of the vitamins had been assayed independently. Because the B-vitamins interact with one another in complex, and not completely defined, ways we should then set up a second series of cultures to each group of which we had added two of the vitamins. Then, mixtures of three vitamins would require examination, and so on. Instead, we have performed the converse experiment; comparing growth in a medium containing all vitamins, to that in a series of cultures each of which contains all of the vitamins with the exception of one.

As can be seen in Table 1, cultures from which riboflavin, niacinamide, thiamine, para-aminobenzoic acid, or pyridoxine were omitted do not differ significantly from those in which all of the vitamins were present. Those cultures from which choline, biotin, or *m*-inositol were omitted grow much more poorly than do those in which all of the vitamins were present. The observed difference can be expected to occur as a result of chance in less than one culture out of 100. When folic acid is omitted from the culture medium, the situation is sommewhat equivocal, that is, the decreased growth might very well occur solely as a result of chance in approximately one-tenth of the cultures.

TABLE 1.

Effect of addition of B-vitamins to medium.

Supplement	No. of Cultures	Mean No. of Free Cells/mm³	t	p
No Vitamins	10	2.7	3.81	< 1 %
AV less Choline	9	5.8	3.58	< 1 %
AV less Biotin	8	28.1	2.63	< 1 %
AV less *m*-Inositol	9	37.9	2.33	< 2 %
AV less Folic Acid	8	52.3	1.71	< 10 %
AV less Riboflavin	10	80.2	< 1	—
AV less Niacinamide	9	96.9	< 1	—
AV (Control)	10	97.8	—	—
AV less Thiamine	8	98.6	< 1	—
AV less PABA	10	117.0	< 1	—
AV less Pyridoxine	10	118.0	< 1	—
AV less Ca-Pantothenate	9	193.0	3.70	< 1 %
Choline alone	10	16.3	3.26	< 1 %
Biotin alone	9	23.4	2.90	< 1 %
m-Inositol alone	7	138.0	1.46	< 20 %
Choline, Biotin, and *m*-Inositol	8	142.6	1.69	< 10 %
AV (Ca-Panthothenate (.001 μg/ml)	10	175.5	3.89	< 1 %

Legend

AV = All B-vitamins listed

p = Probability of these results due to chance alone

$$t = \frac{\text{Error of the Mean}}{\text{Standard Error of the Mean}}$$

Rather surprisingly, cultures from which calcium pantothenate is omitted grow very nearly twice as well as do cultures in which it is present in a concentration of 0.01 μg per ml. This effect of calcium pantothenate becomes somewhat clearer upon examination of growth when the concentration of this vitamin is decreased. When calcium pantothenate is added at 0.001 micrograms per ml. rather than at ten times this concentration, growth is not inhibited.

Since the omission of choline, of biotin, or of *m*-inositol seems to exert such a profound effect on growth, these vitamins were tested by addition of each of them as the only vitamin in a series of cultures. Neither choline alone nor biotin alone is capable of stimulating growth significantly above that of those cultures to which no vitamins were added. As Grace has previously reported, however, the addition of *m*-inositol alone gives results which are approximately the same as those obtained by the addition of all ten vitamins.

The above results do not sharply distinguish between two possibilities. The vitamins which may be omitted from the medium without causing decreased growth may not be essential for the growth of these cells in cultures, but it is equally likely that the results are a consequence of the fact that the explant itself contains a sufficient quantity. In order to decide between these possi-

TABLE 2.

Reported molar inhibition ratio (vitamin/analogue) of complete inhibition of activity.

Vitamin	Analogue	Ratio	Test System	Reference
Biotin	D L Desthiobiotin	1 : 1000000	Growth inhibition (*L. helveticus*)	Robinson
Ca-Pantothenate	D L Pantoyltaurine, Na Salt	1 : 130000	Growth inhibition (*L. arabinosis*)	Robinson
Folic Acid	Aminopterin	1 : 10	Nutritional studies (man)	Robinson
Riboflavin	Atebrine-HCl	1 : 1000	—	—
Niacinamide	3-Pyridine Sulfonic Acid, Na Salt	4 μ moles/ml	Growth inhibition (*P. vulgaris*)	Robinson
m-Inositol	Benzene Hexachloride	1 : 1000	—	—
Thiamine	Oxythiamine chloride	1 : 1000000	Nutritional studies (chickens)	Robinson
p-Aminobenzoic Acid	2 - Chloro - 4 - Amino-benzoic Acid	1 : 30000	Growth inhibition (*E. coli*)	Robinson
Pyridoxine	Deoxypyridoxine	1 : 5	Growth inhibition (T_2r+ *E. coli*)	Robinson

bilities, we have performed a second series of experiments in which an analogue of each vitamin is added to the culture medium while the corresponding vitamin is omitted. Table 2 lists the analogues used, the minimal ratio of analogue-to-vitamin, and a brief reference to previously reported results in which such an analogue-to-vitamin ratio produced inhibition of growth.

Table 3 shows the results of treatment of our cultures with a complete vitamin mixture, with no vitamins, and with a series of mixtures in each of which

one vitamin is replaced by its analogue. In the presence of the analogues of biotin, pyridoxine, para-aminobenzoic acid, and niacinamide, growth is essentially the same as is obtained when no vitamins are added to the medium. (There is less than one chance in a hundred that the addition of all vitamins less one plus the analogue is the same as the addition of all the vitamins). The

TABLE 3.

Effect of simultaneous omission of each vitamin and addition of its analogue.

Supplement	No. of Cultures	Mean No. of Free Cells/mm^3	t	p
No Vitamins	10	2.7	3.81	< 1 %
AV less Biotin plus A	9	2.8	3.71	< 1 %
AV less Pyridoxine plus A	8	6.2	3.45	< 1 %
AV less PABA plus A	10	10.2	3.50	< 1 %
AV less Niacinamide plus A	8	29.3	2.58	< 1 %
AV less Folic Acid plus A	9	35.4	2.43	< 2 %
AV less Riboflavin plus A	8	39.2	2.21	< 5 %
AV less *m*-Inositol plus A	8	44.4	2.01	< 5 %
AV less Thiamine plus A	9	48.2	1.90	< 5 %
AV less Ca-Panthothenate plus A	9	63.0	1.35	< 20 %
AV (Control)	10	97.8	—	—

Legend

AV = All B-vitamins listed   A = Analogue

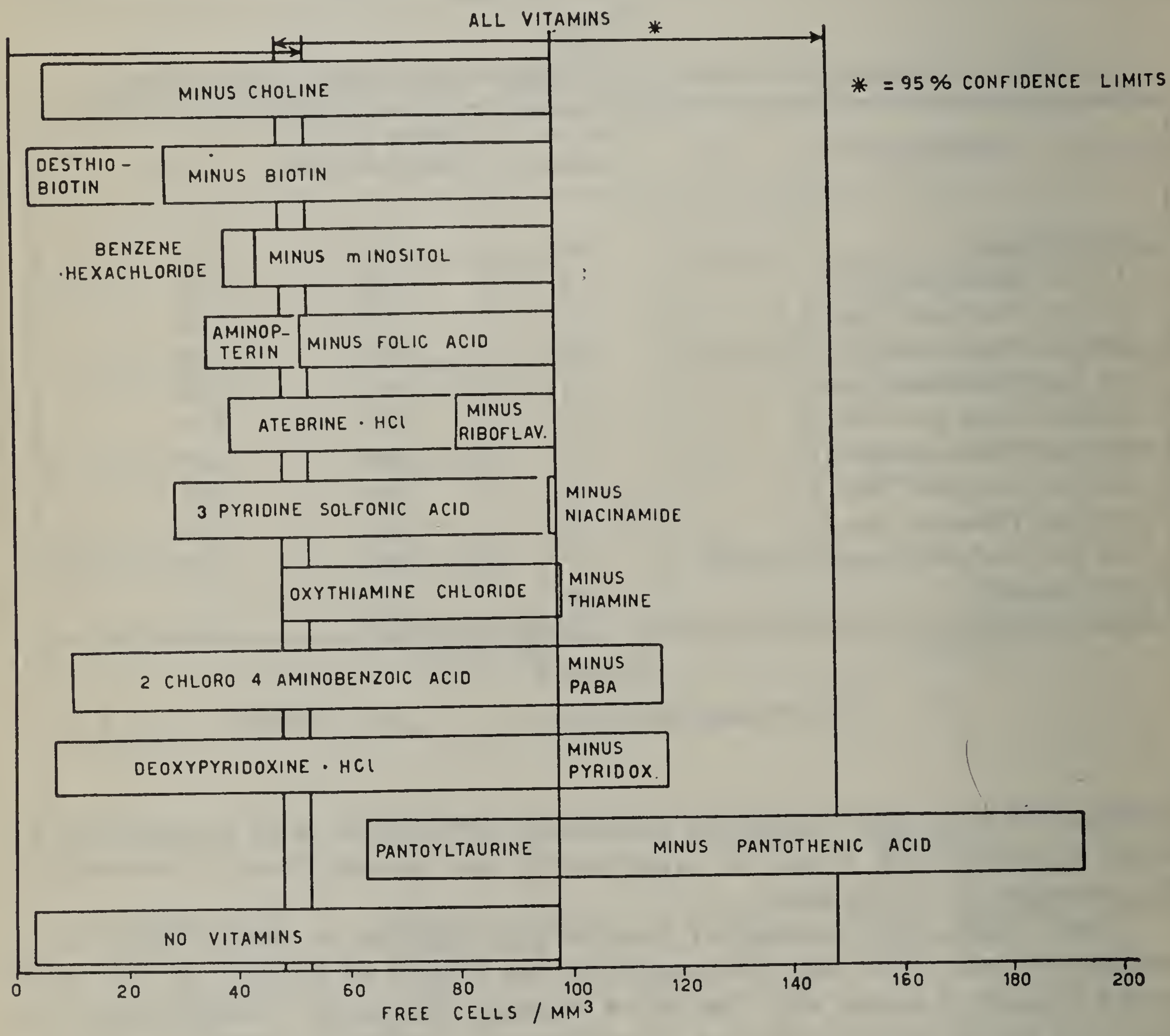
omission of folic acid, riboflavin, *m*-inositol, or thiamine with replacement by their analogues also results in significantly less growth than is obtained in the presence of all vitamins.

Once again, the omission of calcium pantothenate in the presence of its analogue seems to be less detrimental to the growth of cultures at the levels tested than is the case with the other vitamins. The only vitamin which has not been tested for the effect of its analogue is choline for which there is no suitable antimetabolite.

DISCUSSION

On the basis of the experiments reported here, which are summarized in Figure 1, we can say that each of the B-vitamins tested is essential for migration or growth under the conditions we have used, that the explants, the hemolymph, or the adrenal cortical extract contain sufficient folic acid, riboflavin,

niacinamide, thiamine, para-aminobenzoic acid, and pyridoxine to permit optimum growth but all are deficient in choline, biotin, and *m*-inositol. Of these three deficient molecules, the most crucial seems to be *m*-inositol.



In addition, the growth response of these cultures seems to depend upon a critical concentration of pantothenic acid. Since growth is significantly better in the absence of added pantothenic acid than under any other circumstances, we believe that very nearly optimal concentrations of pantothenic acid are present in the explant and that further addition serves only to inhibit growth. A low concentration of pantothenic acid must, however, be essential for tissue growth since the addition of its analogue, pantoyltaurine, inhibits growth.

We have not yet succeeded in obtaining a definitive assay of all of the vitamins studied, in the adrenal cortical extract which we have used. We do know that it contains less than 0.1 micrograms per ml of biotin and less than 20 micrograms per ml of *m*-inositol. It seems unlikely that such levels of these two vitamins can fully explain the observed stimulation by the aqueous adrenal cortical extract. Assuming that the cortical extract contains very slightly less than the amounts of these vitamins which can be detected by the assays used, the addition of 5 ml of cortical extract per 100 ml of culture medium would provide a maximum of 0.005 micrograms of biotin and 1.0 micrograms per ml of *m*-inositol.

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SUMMARY

1. Stimulation of growth and migration of cells from ovaries of pupal *Samia cynthia* by members of the vitamin B-complex has been observed in tissue culture.
2. Judging from the results obtained with analogues of the B-vitamins, insect cells in culture require the presence of choline, biotin, *m*-inositol, folic acid, riboflavin, niacinamide, thiamine, *p*-aminobenzoic acid, and pyridoxine.
3. In the absence of added choline, biotin, or *m*-inositol, growth is very much less than in their presence.
4. Calcium pantothenate at concentrations of 0.01 micrograms per ml decreases growth and migration in these cultures, but in the presence of its analog, pantoyltaurine, growth is inhibited.

RIASSUNTO

Esigenze chimiche per la crescita di tessuti di Insetti in vitro.

1. Nelle colture di tessuto è stata osservata la stimolazione dello sviluppo e la migrazione di cellule degli ovari di pupe di *Samia cynthia* causate da vitamine del complesso B.
2. Secondo i risultati ottenuti con analoghi di vitamine B, le cellule di Insetti in colture richiedono la presenza di colina, biotina, *m*-inositolo, acido folico, riboflavina, niacinamide, tiamina, acido *p*-aminobenzoico e piridossina.
3. Senza aggiunta di colina, biotina, o *m*-inositolo, lo sviluppo è molto più basso che in loro presenza.
4. Alla concentrazione di 0,01 microgrammi per ml di pantotenato di calcio, lo sviluppo e la migrazione in queste colture diminuisce, in presenza del suo analogo pantoil taurina, lo sviluppo è inibito.

SYMPOSIUM 4:

CHEMICAL DEFENSIVE MECHANISMS IN ARTHROPODS
CHEMISCHE VERTEIDIGUNGSMECHANISMEN BEI ARTHROPODEN
MECCANISMI CHIMICI DI DIFESA NEGLI ARTROPODI
MECANISMES CHIMIQUES DE DEFENSE CHEZ LES ARTHROPODES

Symposium 4: CHEMICAL DEFENSIVE MECHANISMS IN ARTHROPODS

Vienna, August 18, 1960

INTRODUCTION

This Symposium, the first of its kind, took place at the Zoological Institute of the University of Vienna, on the afternoon of August 18, 1960. Although the chemical defensive mechanisms of arthropods have been the subject of some interest in the past, it has only been in the last few years that research in this field has taken on an intensive pace. The Symposium has therefore fulfilled a timely function, inasmuch as it has brought together at an early stage many of the investigators involved. It is regrettable that others, whose presence would have greatly added to the success of the meeting, were prevented from attending due to lack of adequate travel funds.

The central topic of discussion was the defensive substances produced by insects and other terrestrial arthropods, and present either in their blood, or secreted by special glands. Some of the papers dealt with the nature of the active principles involved, including the techniques that led to their isolation, purification, and chemical identification. Subjects of a more purely biological nature were also considered, such as the defensive effectiveness of the secretions against predators, the nature of the glands and the operation of the discharge mechanism, the extent to which some of the arthropods are involved as models of mimicry complexes, etc.

In the pages that follow are presented a few short abstracts of some of the work discussed at the symposium. It is hoped that meetings on the same general subject will be held in the future, but perhaps one should chose a broader approach to the problems involved. Rather than restricting oneself to the defensive mechanisms of arthropods, one could consider those of invertebrates and vertebrates as a whole. Also, rather than dealing only with defenses used against predators, one might include the special chemical defenses that protect against systemic infection by microorganisms.

I would like to express my indebtedness to Professor Mario Pavan of the University of Pavia, for having secured the funds that made the prompt publication of these proceedings possible.

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INTRODUZIONE

Questo Simposio, primo nel suo genere, ebbe luogo all'Istituto Zoologico dell'Università di Vienna il pomeriggio del 18 agosto 1960. Benchè i meccanismi chimici difensivi negli Artropodi siano stati un argomento di qualche interesse nel passato, è stato solo in questi ultimi anni che la ricerca in questo campo ha avuto uno sviluppo intensivo. Il Simposio ha perciò compiuto una funzione tempestiva poichè ha riunito in questo stadio iniziale molti dei ricercatori interessati. E' spiacevole che altri, la cui presenza avrebbe grandemente aumentato il successo della riunione, non abbiano potuto parteciparvi per la mancanza dei finanziamenti necessari al viaggio.

L'argomento centrale della discussione è stato quello delle sostanze difensive prodotte dagli Insetti e da altri Artropodi terrestri, presenti nell'emolinfa o secrete da glandole speciali. Alcuni dei lavori trattano la natura dei principi attivi implicati, includendo le tecniche che portarono al loro isolamento, purificazione e identificazione chimica. Furono presi in considerazione anche argomenti di natura più prettamente biologica, ad esempio l'efficacia difensiva delle secrezioni contro i predatori, la natura delle glandole e i meccanismi di utilizzazione, fino a che punto alcuni Artropodi sono implicati nei complessi fatti di mimesi, ecc.

Nelle pagine seguenti sono presentati brevi estratti di alcuni dei lavori discussi al Simposio. Si spera che altre riunioni verranno tenute in futuro sullo stesso argomento generale, ma forse ci si dovrebbe inoltrare maggiormente nei problemi. Piuttosto che limitarci ai meccanismi difensivi degli Artropodi si potrebbero considerare quelli degli invertebrati nell'insieme. Inoltre, piuttosto di trattare solamente difese usate contro i predatori, si potrebbero includere le speciali difese chimiche che proteggono dall'infezione sistemica per opera di microrganismi.

Vorrei esprimere la mia gratitudine al prof. Pavan dell'Università di Pavia per aver procurato i fondi che hanno reso possibile una veloce pubblicazione di questi Atti.

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FRAZER J. F. D., ROTHSCHILD M. (*)

DEFENCE MECHANISMS IN WARNINGLY-COLOURED MOTHS AND OTHER INSECTS

The theory of warning coloration has been generally accepted by entomologists. Recently however, we had cause to question the reason why predators do not accept these visibly protected species. While handling two of the common Arctiid moths, one of us experienced severe stings. It then occurred to us that these warningly coloured insects, which also specialise in warning displays, might possess some unsuspected chemical defence mechanism and contain in their tissues toxins which had hitherto passed unnoticed. We approached the problem from two angles.

a) We set up a series of ' screening ' tests with the aid of caged predators which included such animals as various insectivorous mammals and birds, and also lizards, terrapins and toads. By this method we were able to sort out the most unacceptable species of several hundred different insects.

b) We carried out a series of tests to detect, if possible, the presence of pharmacologically active principles, such as histamine, acetylcholine and 5-hydroxytryptamine, which are frequently associated with venoms, venomous tissues or stinging organs in widely different species of animals and plants.

In the prothoracic (cervical) glands of the moth *Arctia caja* we were able to show (Bisset, Frazer, Rothschild and Schachter, 1960) the presence of β, β-dimethylacrylylcholine or some closely allied choline ester. This ester has interesting pharmacological properties. It can stimulate the nerve ganglia of mammals (Keyl, Michaelson and Whittaker, 1957) while according to Dubois (1909) it causes muscular paralysis in both warm- and cold-blooded animals.

The secretions of the prothoracic glands do not, however, contain the only substances of pharmacological interest in the Garden Tiger moth. Thus we have found that extracts from the terminal segments of the abdomen of the female (but not of the male) are rapidly lethal to guinea-pigs when given intravenously. This work has been taken further by Parsons and Paton (1960), who have shown that a toxic substance is also present in the eggs of the moth, which is equally

(*) *The Nature Conservancy, London, and Ashton, Peterborough, England.*

rapidly lethal to cats. They also discovered another choline ester in the abdomen of the males of *Arctia caja* which we had failed to detect (Frazer and Rothschild, 1959).

Preliminary investigations of the closely-related Cream-spot Tiger moth (*Arctia villica* L.) by Parsons and Paton (1960) show that there are similar

TABLE 1

Mean rating of unacceptable insects fed to caged predators

Insect species have been given ratings from O (fully acceptable) to 7 (totally unacceptable) based on trials against all the predators listed here. In addition to the species given, many other insects were tested to a total of several hundred, but owing to seasonal difficulties were not offered to all predators.

A. PREDATORS ON WHICH TESTS WERE MADE

BIRDS :	Shama (*Kittacincla malabarica* Gm.)
	Sibia (*Lioptila capistrata* Vig.)
	House Sparrow (*Passer domesticus* L.)
	Great Tit (*Parus major* L.)
	Himalayan Crested Tit (*Machlolophus xanthogenys* Vig.)
	Cuckoo (*Cuculus canorus* L.)
MAMMALS:	House Mouse (*Mus musculus* L.)
	Bush Babies (*Galago crassicaudatus* Geoffroy; *G. moholi* Smith)
	Short-tailed Field Vole (*Microtus agrestis* L.)
	Long-eared Bat (*Plecotus auritus* L.)
	Pipistrelle Bat (*Pipistrellus pipistrellus* Schreber)
	Hedgehog (*Erinaceus europaeus* L.)
REPTILES :	Lizards
	Terrapins
AMPHIBIA:	Toad (*Bufo bufo* L.)

B. ACCEPTABILITY RATINGS

Ladybirds (*Coccinella 7-punctata* L., *C. 11-punctata* L. and *Adalia bipunctata* L.)	7
Froghopper (*Cercopis vulnerata*) (Ger.)	7
Cinnabar moth (*Hypocrita jacobaeae* L.); all stages	7
Scarlet Tiger moth (*Panaxia dominula* L.)	6
Soldier Beetle (*Rhagonycha fulva* Scop.)	6
Wasp (*Vespula vulgaris* L.)	5-6
Burnet moths (*Zygaena lonicerae* von Schev. and *Z. filipendulae* L.)	5
Magpie moth (*Abraxas grossulariata* L.)	5
Small Magpie moth (*Eurrhypara hostulata*) (L.)	5
Common Footman moth (*Eilema griseola* Hbn.)	5
Garden Tiger moth (*Arctia caja* L.)	4
Ruby Tiger moth (*Phragmatobia fuliginosa* L.)	4
Gold-tail moth (*Euproctis chrysorrhaea* L.); Male:	4
Female:	5
Small White butterfly (*Pieris rapae* L.)	4
Green-veined White butterfly (*Pieris napi* L.)	4
Large White butterfly (*Pieris brassicae* L.)	4
White Ermine moth (*Spilosoma lubricipeda* L.)	4
Buff Ermine moth (*S. lutea* Huf.)	3

substances in this species with the addition of a depressor substance (possibly adrenaline) in the abdomen. The smell from the prothoracic (cervical) glands of this moth is the only one from any moth so far tested which appears to the human nose identical with that of the Garden Tiger.

The species of moth most unacceptable to predators among those which we tested was the Cinnabar (*Hypocrita jacobaeae* L.). This insect was refused by seven species of insectivorous mammals (including the hedgehog) and five species of insectivorous birds, as well as lizards and terrapins. It was less acceptable than either the Five-spot Burnet (*Zygaena lonicerae* von Schev. and *Z. filipendulae* L.) or Scarlet Tiger (*Panaxia dominula* L.) moths. In the Cinnabar moth (*Hypocrita jacobaeae* L.) Bisset *et al* (1960) found a very high histamine content of the body tissues (750 mg/g of freezedried body tissue).

The relationship between warning colour, histamine content and acceptability is not clear. Histamine is certainly found in many of the species we tested on account of their high unacceptability rating (see Table 2) such as the Cinnabar (*Hypocrita jacobaeae* L.), Five-spot Burnet (*Zygaena lonicerae* von Scheven), White Ermine (*Spilosoma lubricipeda* L.), Magpie (*Abraxas grossulariata* L.) and Gold-tail (*Euproctis chrysorrhoaea* L.) moths, and Ladybird (*Coccinella* sp. L.), but only traces of it were detected in the Scarlet Tiger (*Panaxia dominula* L.), Garden Tiger (*A. caja* L.) and Buff Ermine (*S. lutea* Huf.). The Scarlet Tiger is highly unacceptable (rating 6) and the Buff Ermine with a 2-3 rating is only slightly more favoured in paired feedings with the White Ermine, although the White Ermine as we have seen is loaded with histamine (700 mg/g of body tissue). It seems possible that high histamine levels are associated with some toxin (in conjunction with which it has a special function as in the nettle sting) which is at present not clearly understood. Histamine affects the gastric secretion in man, but its effect on birds or upon invertebrates is not known. Lane and Rothschild (1957) first noted that the female Gold-tail (*Euproctis chrysorrhoaea* L.) is less acceptable to certain bird predators than the male, even when the hairy tails have been removed prior to feeding. Parsons and Paton (1960) have found that there is seven to eight times as much histamine in the female (100 mg/g of the whole moth) as in the male. Both sexes are, however, less acceptable to birds than the White Ermine; the latter is invariably chosen and is unquestionably relatively more acceptable to bats as well as birds. Histamine alone cannot therefore be the cause of birds refusing certain moths as food.

The presence of toxins in the bodies of these moths, apart from histamine, is suggested by Rocci's (1917) and Rothschild's (in Lane and Rothschild, 1959) experiments with the Five-spot Burnet (*Zygaena trifolii* Esper).

The Ladybirds (*Adalia bipunctata* L. and *Coccinella septempunctata* L.) are also highly unacceptable insects (with a rating of 7). One specimen ground up and injected (i. v.) into the guinea-pig proved rapidly fatal. Whether this effect is due only to the high histamine content of the Ladybird tissues is not known, but this is thought improbable since the Ladybird contains 150 mg. per

gram of body tissue and the Burnet (*Z. lonicerae*) 250 mg. The latter species, although considerably larger than a single Ladybird, does not prove fatal to the guinea-pig when injected intravenously, and one consequently suspects the presence of some other toxin in the Ladybirds. It should also be noted that the

TABLE 2

	Moths					
	Garden Tiger *Arctia caja*	Ruby Tiger *Phragmatobia fuliginosa*	Cinnabar *Hypocrita jacobaeae*	Scarlet Tiger *Panaxia dominula*	Cream Spot Tiger *Arctia villica*	5-sp Bur *Zyg lonic*
Warning Colour						
Red	+	+	+	+	+	+
Yellow	—	—	—	+	+	—
Black	—	—	+	—	+	—
White	+	—	—	+	—	—
Distinctive smell . . .	+	+	+	+	+	—
Distasteful to birds and bats	rating 4 ++++	rating 4 ++++	rating 7 +++++++	rating 6 ++++++	not known	ratin +++
Can sting	+	+	—	—	—	—
Histamine tests						
Present (Bisset *et al.* 1960)	traces?	traces?	+++	—	—	+
Histamine-like effects						
Guinea-pig ileum . . .	—	—	+	+		—
Guinea-pig skin	+	+		+		—
Acetylcholine-like activity	+				+	

(*) See Jacques & Schachter, 1954.

Scarlet Tiger (which has an unacceptability rating of 6) apparently has neither histamine nor acetylcholine-like activity in its tissues. It nevertheless produces quite a disagreeable local irritation of the throat if chewed up by man (Lane,

1958), and the volatile substances from the prothoracic glands produce asthma in certain subjects.

The problem is an exceedingly complex one since, quite apart from the question of toxins which may act upon the internal organs, factors such as

TABLE 2

...ff ...ine ...oma ...a	White Ermine *Spilosoma lubricipeda*	Magpie *Abraxas grossulariata*	Small Magpie *Eurrhypara hortulata*	Gold-tail *Euproctis chrysorrhaea*	Ladybird *Coccinella* sp.	Soldier Beetle *Rhagonicha fulva*	Wasp *Vespula vulgaris*
	Moths						
	— + + +	— + + +	— + + +	— + — +	+ — + —	+ — — —	— + + —
	+	—	—	+	+	+	—
...; 3 ...+	rating 4 ++++	rating 5 +++++	rating 5 +++++	rating 1 +	rating 7 +++++++	rating 7 +++++++	rating 1 +
	—	—	—	—	—	—	+
	+++						+ (*)
	+ +	+	+	+	+	+	+ + (*)
				—			

disagreeable taste and local irritation of the buccal cavity, and also smell, probably play an important part in determining unacceptability. It is clear that animals have very varied and specialised feeding habits and illustrate perfectly

the proverb that 'one man's meat is another man's poison'. This is well shown by the example of the Small White butterfly (*Pieris rapae* L.). The Long-eared Bat (*Plecotus auritus* L.), which in captivity is a voracious feeder upon Lepidoptera, from Red Admiral butterflies (*Vanessa atalanta* L.) to all the British Hawk moths, will not touch this species under any circumstances, short of actual starvation. Nor will the equally voracious Shama (*Kittacincla malabarica* Gm.). The Himalayan Crested Tit (*Machlolophus xanthogenys* Vig.) and the Sibia (*Leoptila capistrata* Vig.) on the other hand eat them eagerly and in vast numbers. Again, the Shama will devour the Green-bottle fly (*Lucilia* sp.) which is persistently refused by the Tits. The only species so far refused by all caged predators is the Cinnabar, which is even rejected by the Short-tailed Field Vole

TABLE 3

Unacceptability ratings 7 - 0 relative to different species

	Long-eared Bats	Field Voles	Tits	Shama
CINNABAR moth (*Hypocrita jacobaeae* L.)	7	7	7	7
BURNET moth (*Zygaena lonicerae* von Schev. and *Z. filipendulae* L.)	7	2	5	5
SCARLET TIGER moth (*Panaxia dominula* L.)	7	?	6	6
SMALL WHITE butterfly (*Pieris rapae* L.)	7	0	0	7
COMMON FOOTMAN moth (*Eilema griseola* Hbn.)	7	0	4	0
MOTHER-OF-PEARL moth (*Notarcha ruralis* Scopoli)	7	0	4	0
GOLD-TAIL moth (*Euproctis chrysorrhoaea* L.)	5	0	2	3
RED ADMIRAL butterfly (*Vanessa atalanta* L.)	0	0	0	0

and Hedgehog, which, on the other hand, eat the Burnet refused by all our other caged predators. These examples could be multiplied.

To sum up, it seems highly probable that other species with aposematic colouring, apart from the Garden and Creamspot Tigers, have toxins present in their body tissues. But these toxins may be very varied, numerous and difficult to isolate and identify. Their action upon the various predators may be equally varied. The whole story will obviously take a lot of unravelling, and may be further complicated by the occurrence of Mullerian mimicry, but it would appear that we are on the fringe of a vast but interesting new field relating to the chemical defences of the Lepidoptera.

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SUMMARY

The emission of a strong smelling fluid from the specialized defensive glands of insects is often associated with an aposematic colour scheme and, in moths, the ability to produce a warning display. Experiments have been conducted principally on certain British Lepidoptera to determine whether this fluid contains toxic properties. In the case of *Arctia caja* (L.) (*Lepidoptera*: *Arctiidae*) it was found to contain a choline ester, akin to and probably identical with ββ-dimethylacrylylcholine. It has also been found that the body of *A. caja* and *Coccinella septempunctata* L. (*Coleoptera*: *Coccinellidae*) contain substances lethal to guinea pigs when injected intravenously, which apparently act by the release of histamine. A marked difference between the lethal properties of the substances found in the bodies of the male and female of *A. caja* has been demonstrated.

Histamine is also found in heavy concentrations in a variety of aposematic moths such as *Hypocrita jacobaeae* (L.), *Zygaena lonicerae* (von Sch.), *Spilosoma lubricipeda* (L.), *Abraxas grossulariata* (L.), and *Eurrhypara hostulata* (L.). It is not known whether this substance plays any part in their defence mechanism, but its distribution among the moths so far examined is suggestive.

RIASSUNTO

Meccanismi difensivi in Insetti colorati con colori premonitori.

L'emissione di un liquido fortemente odoroso da speciali glandole difensive di Insetti è spesso associato a uno schema colorato aposematico e, in Lepidotteri, alla capacità di produrre un segno di allarme. Sono stati fatti esperimenti principalmente su certi Lepidotteri inglesi per determinare se la secrezione contenga proprietà tossiche. Nel caso dell'*Arctia caja* (L.) (*Lep. Arctiidae*) fu trovato che essa contiene un etere della colina affine e probabilmente identico a ββ-dimetilacrililcolina. Si è anche scoperto che i corpi di *A. caja* e *Coccinella septempunctata* L. (*Col. Coccinellidae*) contengono sostanze letali alla cavia (*Cavia cobaya*), quando sono iniettate per endovena, che agiscono apparentemente come istamino-liberatrici. E' stata dimostrata una notevole differenza fra le proprietà letali delle sostanze trovate nei corpi dei maschi e delle femmine di *A. caja*.

Si trova anche istamina in alte concentrazioni in Lepidotteri aposematici come la *Hypocrita jacobaeae* (L.), *Zygaena lonicerae* (von Sch.), *Spilosoma lubricipeda* (L.), *Abraxas grossulariata* (L.) e *Eurrhypara hostulata* (L.). Non si sa se questa sostanza prenda parte al meccanismo di difesa, ma la sua distribuzione fra i Lepidotteri esaminati è interessante.

ROTHSCHILD MIRIAM (*)

DEFENSIVE ODOURS AND MULLERIAN MIMICRY AMONG INSECTS

SUMMARY

It has been remarked that various aposematic insects from several different orders including *Lepidoptera*, *Coleoptera* and *Hemiptera* have defensive odours which resemble each other. It is suggested that this is an example of Mullerian mimicry — the scent patterns acting for colour blind predators in the same manner as the well known warning patterns of red and black, and yellow and black rings etc. act in the case of predators (such as birds and entomologists) which hunt by sight. A technique has been evolved for examining these odours by means of gas chromatography.

RIASSUNTO

Odori difensivi e mimetismo mulleriano negli Insetti.

E' stato notato che parecchi Insetti aposematici di differenti Ordini comprendenti *Lepidoptera*, *Coleoptera* ed *Hemiptera*, hanno odori difensivi che si assomigliano. Si presume che questo sia un esempio di mimetismo mulleriano: i modelli profumati agirebbero per i predatori ciechi ai colori nello stesso modo dei ben noti modelli d'avviso a cerchi rossi e neri, gialli e neri, ecc. nel caso dei predatori (ad es. uccelli, entomologi) che catturano a prima vista. Si è sviluppata una tecnica per esaminare questi odori a mezzo del gas-cromatografo.

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BROWER L. P., VAN ZANDT BROWER J. (*)

EXPERIMENTAL STUDIES OF MIMICRY: REACTIONS OF TOADS TO BUMBLEBEES AND THEIR ASILID-FLY MIMICS

Experiments were done with caged toads (*Bufo terrestris*) as predators and bumblebees (*Bombus americanorum*), the models, and asilid-flies (*Mallophora bomboides*), the mimics, as prey. The experimental toads learned to reject bumblebees on sight alone after trials in which they were apparently stung. They then also rejected the flies on sight. The control toads, which never had bees, ate the asilid-flies to a significantly ($P < .001$) greater extent than the experimental toads. A film was made that shows the models and mimics in their natural environment and laboratory reactions of an experimental toad to a palatable non-mimetic insect, to a bumblebee, and to an asilid-fly. It then shows the reactions of a control toad to a palatable non-mimetic insect, to an asilid-fly, and finally to its first bumblebee.

RIASSUNTO

Studi sperimentali sul mimetismo: reazioni di Rospi verso i Bombi e i loro mimetici Ditteri Asilidi.

Furono fatti esperimenti con Rospi (*Bufo terrestris*) come predatori, Bombi (*Bombus americanorum*) come modelli ed i loro mimetici Ditteri Asilidi (*Mallophora bomboides*) come preda. I Rospi sperimentali impararono a rifiutare i Bombi a prima vista solo dopo prove in cui furono punti. Poi essi respinsero gli Asilidi a prima vista. I Rospi di controllo che non furono mai punti, mangiarono gli Asilidi, in modo significativo (P .001), superiore ai Rospi sperimentali. Un film mostra i modelli e i mimetici nell'ambiente naturale e reazioni di laboratorio di un Rospo verso un Insetto non mimetico gradito come cibo, verso un Bombo e verso un Asilide. Vi si mostra anche la reazione di un Rospo di controllo verso un Insetto non mimetico gradevole al palato, verso un Asilide e infine verso il suo primo Bombo.

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EDWARDS J. S. (*)

SPITTING AS A DEFENSIVE MECHANISM IN A PREDATORY REDUVIID

The chemical defence mechanism to be discussed in this paper is unusual in being a means of offence against insects that serves also as a defence against vertebrates. It is a mechanism that makes use not of special dermal gland secretions, nor secretions of aboral origin, but of the saliva, which in most Heteroptera, certainly in all predatory Heteroptera, plays an important role in feeding. There are at least 2500 species of predatory Heteroptera, but the defensive mechanism I shall describe is so far known only from one assassin bug, *Platymeris rhadamanthus* Gerst., a large reduviid that occurs in East Africa and Zanzibar. Vanderplank (1958) has recorded its range of prey in the Zanzibar coconut plantations, and notes that when disturbed it will eject a stream of liquid from its rostrum. I shall refer to this habit as ‘ spitting behaviour ’.

The following observations were made on material from a laboratory culture originating from specimens kindly sent from Zanzibar by Dr. F. L. Vanderplank.

When disturbed, either physically or by sudden movement in the vicinity, particularly when associated with a rapid change in incident light, the animal may be stimulated to ejaculate saliva. It remains stationary and alters its stance so that the body is raised on one side and lowered on the other, the head is rotated slightly on the longitudinal axis and the penultimate segment of the rostrum is deflected so that the rostral tip is directed over and to one side of the body. Accuracy in aiming the saliva toward a source of disturbance is achieved by deflecting the terminal segment of the rostrum as each jet is ejaculated. The lateral retractor muscles of both these rostral segments are well developed, and the segments are mobile.

Brief stimulation of the animal elicits one or two spits, but a highly excited animal may deliver as many as 15 successive spits at a rate of 3 - 5 per second.

The saliva is ejaculated with considerable force, and will travel as far as 30 cm. Figure 1 shows the spray pattern produced by an adult *Platymeris*

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rhadamanthus. It is a contact print from a glass plate held 3 cm. above an animal that was touched with a probe first on one wing then on the other. The saliva was fixed with absolute alcohol and stained with eosin. A series of spits on either side of the mid line can be seen. An outline of the animal in position below the glass, and an indication of the ' arc of fire ' – ca. 65° – achieved by deflecting the rostrum is also shown. The largest single ejaculation obtained by

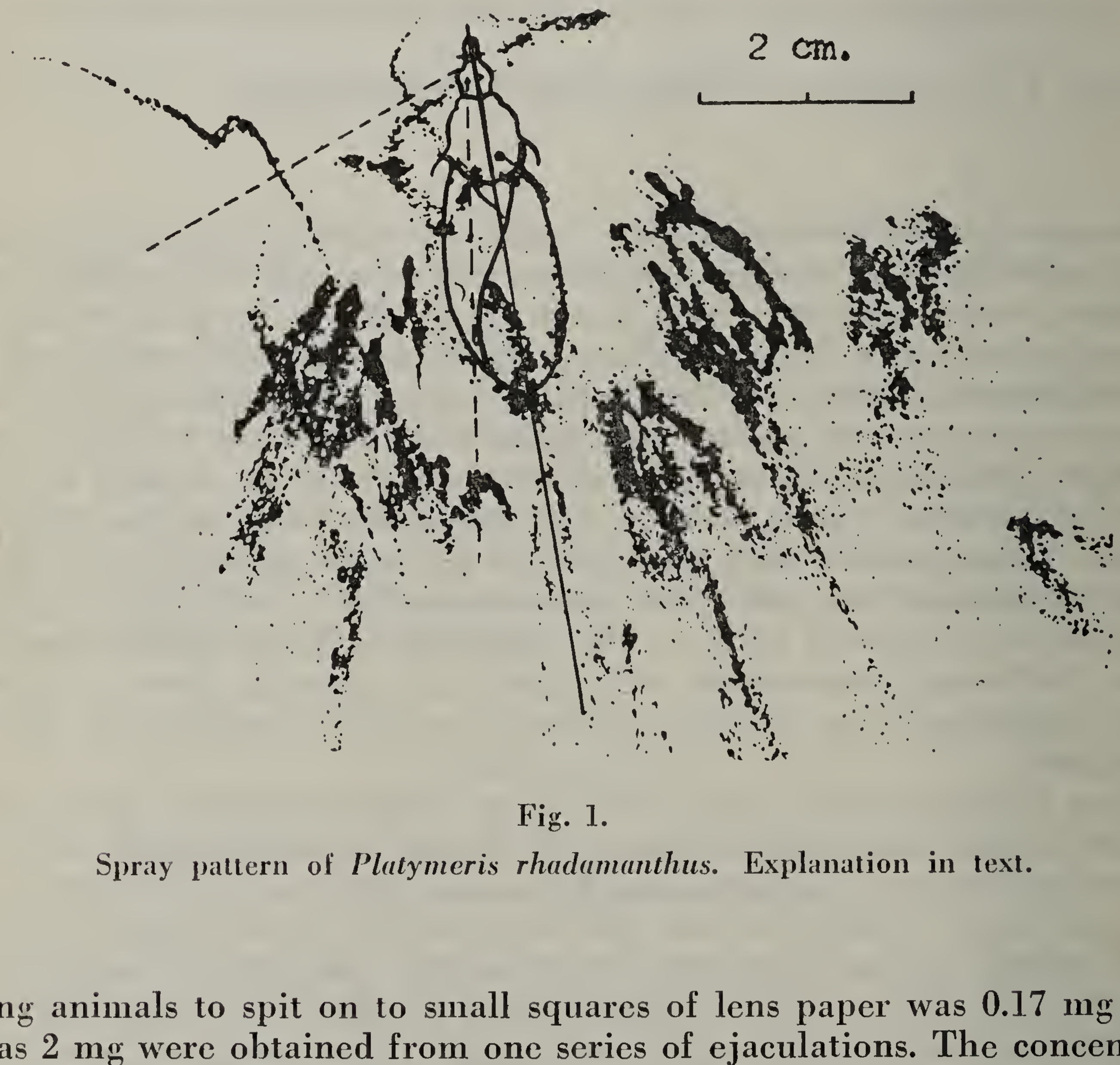


Fig. 1.

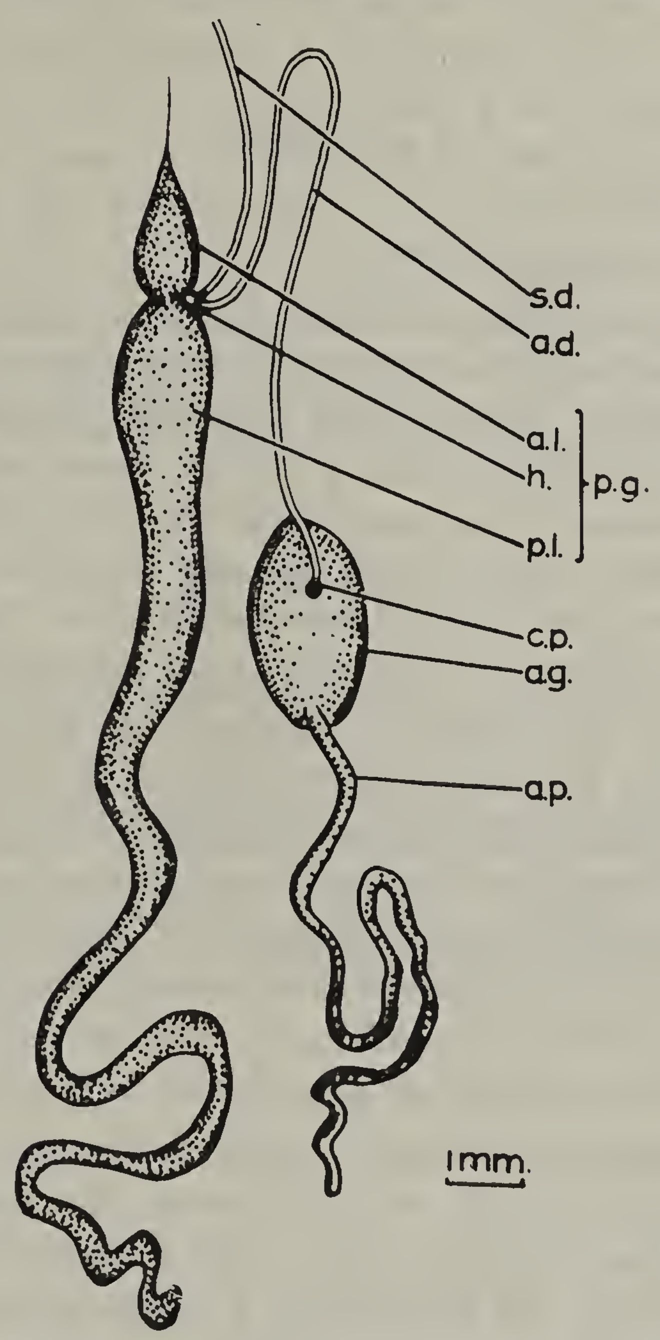
Spray pattern of *Platymeris rhadamanthus*. Explanation in text.

inducing animals to spit on to small squares of lens paper was 0.17 mg and as much as 2 mg were obtained from one series of ejaculations. The concentration of salivary solids varied, according to the state of dehydration of the animal, between 9 % and 20 %, the largest quantity of dry material obtained at one time being 0.96 mg.

Ejaculation is achieved by means of the salivary pump. This structure has been described in several Heteroptera (e. g. Weber 1936; Barth 1952). It need only be noted that the pump is relatively large in *Platymeris* and is filled by means of a massive retractor muscle which effects the inspiratory stroke. The power for the return stroke which ejects the saliva depends on the elasticity of the outer wall which is composed of rubber-like cuticle of the type described by Weis Fogh (1959) in insect flight muscle tendons. Saliva reaches the exterior

via the stylet canal which in *Platymeris* has a functional length of ca. 8 mm, and an average diameter of 65μ. As in other reduviids it is the 'food canal' greatly enlarged at the expense of the salivary canal, that carries both the ejaculated saliva out and the ingested food back.

The active components of the saliva are secreted in the anterior and posterior lobes of the principal salivary gland. The accessory gland secretion, a



Salivary glands of *Platymeris rhadamanthus*.

a.d. accessory gland duct
a.g. accessory gland
a.l. anterior lobe
a.p. process of accessory gland
c.p. cuticular plate
h. hilus
p.g. principal gland
p.l. posterior lobe
s.d. salivary duct

watery material lacking the viscid protein constituents of the principal gland, is non toxic. Saliva collected from animals by exploiting the spitting behaviour causes prompt immobilization when introduced into the insect haemocoel. Details of the action of the saliva are described elsewhere (Edwards 1961). The major effects are muscular contracture followed by relaxation, a transient burst of central nervous activity leading to electrical silence, followed by general lysis, in which the fat body and muscular tissue rapidly break down. *Periplaneta* heart preparations were brought to a standstill within 60 seconds at a concentration of 10^{-6}. Topical and enteral applications have little or no action; indeed cochroaches will drink a 1 % solution of saliva and *Calliphora* larvae survive 12 hours in contact with a 1 % solution of the saliva: the saliva must enter the haemocoel to exert its action.

Vertebrates on the other hand are exceedingly sensitive to the salivary toxin. The bite of the assassin bug is extremely painful: there are several accounts in the literature of effects, varying from localised pain and ' blisters ' to paralysis and necrosis. Contact of spat saliva of *Platymeris* with eye and nose membranes causes intense local pain, vasodilatation and oedema, all symptoms of histamine action. Periodic contact with the dust of saliva during routine collections led to severe bronchial disturbance, similar to that reported by Stanic (1956) as a result of collecting dried venom of the snake *Vipera ammodytes.*

The composition of the saliva is discussed in detail elsewhere (Edwards 1961). The toxic activity lies in the non-dialysable protein fraction. At least 6 proteins are present, three of which show trypsin-like proteolytic activity. The saliva has strong hyaluronidase activity, as shown by its viscosity-reducing action on synovial fluid hyaluronate-complex. It also has weak phospholipase activity. Lipase, esterase and cholinesterase activity were not detected, nor were two common venom constituents, ATP-ase, and serotonin. The saliva resembles snake venom both in number of proteins and in its enzyme activity.

The action on mammalian tissue suggests histamine liberation and neurotoxic activity due to cell lysis. It may be noted that both snake and assassin bug venoms are of salivary gland origin. The parallel indeed goes further in the case of *Naia nigricollis*, the Blacknecked or Spitting cobra of Africa which ejaculates saliva, allegedly to a distance of 2.5 metres by contraction of temporal muscles, and is thus capable of blinding prey.

Taking into account the immunity of insects to topical application of *Platymeris* saliva, and the sensitivity of vertebrate tissues, it would seem that the protective reaction is directed toward vertebrate predators, reptiles, birds and mammals, particularly monkeys. The warning coloration of *Platymeris*: two blood red patches on the wings, the remainder of the animal jet black, supports this view.

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SUMMARY

The behaviour and structures involved in a defensive mechanism of the predatory reduviid *Platymeris rhadamanthus* Gerst., involving the ejaculation of toxic saliva are described. Saliva secreted by the two lobes of the principal gland has an action resembling that of snake venom. Since Arthropods are unaffected by tropical application of the saliva ejaculated by *Platymeris*, but vertebrate eye and nose membranes are highly sensitive, it is concluded that in nature the protective mechanism is directed against vertebrate predators.

RIASSUNTO

Lo sputo come meccanismo di difesa nei Reduvidi predatori.

Sono descritti il comportamento e le strutture implicate in un meccanismo difensivo dell'Eterottero Reduvide *Platymeris rhadamanthus* Gerst., che impiega l'eiaculazione di saliva tossica. La saliva secreta dai due lobi della glandola principale ha un'azione che assomiglia a quella del veleno di un serpente. Poichè gli Artropodi sono insensibili all'applicazione locale della saliva eiaculata da *Platymeris*, mentre le membrane dell'occhio e del naso dei Vertebrati sono altamente sensibili, si è concluso che in natura il meccanismo protettivo è diretto contro i predatori vertebrati.

Eisner T. (*)

THE EFFECTIVENESS OF ARTHROPOD DEFENSIVE SECRETIONS (1)

The defensive glands of terrestrial arthropods have been the subject of some interest in the past, and one may find in the older literature a wealth of information on the kinds of millipedes, arachnids, and insects that produce secretory discharges when handled or otherwise disturbed. More recently, considerable chemical work has been done on these secretions, and the active components have already been identified successfully in several cases. What seems to have been neglected, has been a rigorous study of the glands from the functional point of view. Although they are generally believed to serve in defense, the crucial evidence in support of this view usually seemed to be lacking, since predator-prey encounters had almost never been observed. During the past two years we have done extensive behavioral, physiological, and chemical work on a large array of arthropods that possess defensive glands. The purpose of this short abstract is to summarize only that portion of the work dealing with an evaluation of the defensive effectiveness of the glands. Some of this work has already been published (Eisner 1958 a, 1958 b, Eisner and Blumberg 1959, Eisner et al. 1959), but most of it is forthcoming.

THE DISCHARGE MECHANISM

The number, position, structure, and mode of operation of the glands is extraordinarily diverse, and there is no question that such glands have arisen many times independently in the course of evolution. Some arthropods possess only a single gland, many have one pair, and others, such as most millipedes, have them widely distributed over the entire body. Sometimes true glands do not even seem to be involved: « reflex-bleeding » in coccinellids, for instance, consists in the release of what is apparently pure blood from the tibio-femoral joints of the legs.

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There are two alternative ways in which the secretion can be discharged: as a liquid ooze, and as a spray. Oozing is characteristic, for instance, of many millipedes, some coccinellid beetles, and a variety of tenebrionid beetles. Among those that spray we have worked with representatives of the following: whip-scorpions, phalangids, cockroaches, earwigs, walking sticks, coreid and pentatomid hemiptera, tenebrionid beetles, notodontid caterpillars, ants, and others. There exists considerable variation in the range as well as in the droplet size of the spray. Some discharge a very fine mist, while in others the spray is relatively coarse. Some can spray as far as one meter, others no further than a few millimeters. As a rule, only direct traumatic stimulation will elicit a discharge. In a few cases, however (involving a coreid hemipteran and a walking stick), we have seen the animals discharge in response to movement and/or vibration generated at a distance: birds were sprayed when landing near the animal, before any sort of direct attack had occurred.

An additional finding was that virtually all of the arthropods that spray can control to some extent the direction in which the discharge is ejected, aiming it toward the particular body region subjected to attack. Some can aim with remarkable precision, and in almost all directions (e. g. whipscorpions, bombardier beetles, certain earwigs). Others are considerably less accurate (e. g. certain cockroaches, some tenebrionid beetles). The way in which aiming is accomplished varies considerably. In some cases the gland openings are situated on special revolvable emplacements that can be pointed in different directions. In others there are no such special devices, but accurate aiming is still possible by bending and turning the body as a whole. Additional insurance against wasteful expenditure of secretion is often provided by the tendency to discharge from only those glands that are closest to the stimulus. This held true also for many arthropods that discharge an ooze rather than a spray. In millipedes, for instance, the discharge is usually restricted to the particular segments stimulated, and in coccinellid beetles the release of blood is often localized, and does not come from all legs at once.

EFFECT OF THE SECRETIONS ON PREDATORS

We have observed in the laboratory, as well as under more of less natural conditions in the field, a great many predator-prey encounters involving arthropods with chemical defense mechanisms. The predators tested included ants, carabid beetles, preying mantids, dragon flies, lycosid and orb-weaving spiders, tarantulas, solpugids, centipedes, scorpions, frogs and toads, lizards, birds, grass-hopper mice, armadillos, skunks, opossums, monkeys, and others.

From the experiments it became obvious that the secretions constitute defensive resources of extraordinary effectiveness. Most secretions proved generally repellent to invertebrates and vertebrates alike, there being almost no cases in which a clear-cut group-specific repellency could be demonstrated. The ability of some of the animals to aim the spray was found to enhance the efficacy

of the weapon, especially as it is used against smaller predators that might otherwise be missed.

Of interest also was the fact that the secretion is often ejected with such promptness as to prevent the predator from inflicting disabling injury. This was particularly true when the predators concerned were small: we have never seen any of our arthropods injured by ants as long as they had a supply of secretion. Vertebrates often inflict serious injury before being repelled, but it is interesting to note in this connection that in several instances such vertebrates were shown to have learned to avoid the prey after a few encounters, discriminating against it on sight alone. Equally interesting were the results with grasshopper mice and certain quinone-secreting beetles of the genus *Eleodes*. Although the quinones as such are repellent, the mice can effectively subdue the beetles by holding them head-up and forcing the beetle's abdominal tip downward into the soil, thereby causing the secretion to be ejected into the substrate.

The effects of the secretion on predators — with very few exceptions — are of short-term duration. Following a discharge, the predator releases its hold and flees instantly. Cleansing activities, as well as more or less conspicuous signs of distress were frequently apparent. The duration of such aftereffects depended on the nature of the secretion, the type of predator involved, and the degree of vulnerability of the particular body region hit by the secretion. Recovery was usually complete in a matter of a few minutes. More prolongued effects were rare. Only in exceptional cases is the secretion known to be fatal: the secretion of nasute termites and that of *Iridomyrmex* ants is known to kill some insects (Ernst 1959, Pavan 1951). We are, of course, not dealing here with the venoms produced by many insects and arachnids that are known to be fatal to their victims when injected.

Most of the secretions undoubtedly exert their effect by acting as chemical irritants. Sometimes, however, when they are viscous and sticky, they may also have a direct mechanical effect, particularly on small arthropods. Such an effect was demonstrated for the cephalic exudate of termite nasutes (Ernst 1959, confirmed by myself), and also for the droplets of blood released at the knee joints of coccinellid beetles (Happ and Eisner, unpublished).

Details of all the preceeding, as well as of our chemical and physiological work on the nature of the secretions and their mode of action, will be presented at length in a series of papers.

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SUMMARY

A brief summary is presented on work done on the effectiveness of arthropod defensive secretions. The animals studied included whipscorpions, phalangids, millipedes, cockroaches, earwigs, walking sticks, coreid and pentatomid hemiptera, tenebrionids, coccinellids, notodontid caterpillars, ants, and others. The glands involved, and the mechanisms whereby the secretions are discharged, are extraordinarily diverse. In some, the secretions are produced as a liquid ooze, in others as a jet-like spray. The spray is usually animed accurately in different directions. The effect of the secretions on predators is discussed. A large array of invertebrates and vertebrates was tested, and the secretions, as a rule, proved to be defensive resources of extraordinary effectiveness.

RIASSUNTO

Efficacia delle secrezioni difensive di Artropodi.

Breve riassunto del lavoro fatto sull'efficacia delle secrezioni difensive negli Artropodi. Gli animali studiati includono: Pedipalpi, Opilionidi, Miriapodi, Blattoidei, Dermatteri, Fasmidi, Emitteri Coreidi e Pentatomidi, Coccinellidi, larve di Notodontidi, Formiche, ecc. Le glandole implicate, ed i meccanismi di emissione delle secrezioni, sono straordinariamente diversi. In alcuni, le secrezioni sono prodotte sotto forma di poltiglia liquida, in altri, sotto forma di uno spruzzo. Lo spruzzo è solitamente diretto accuratamente in differenti direzioni. E' discusso l'effetto delle secrezioni sui predatori. Fu esaminato un numero imponente di Vertebrati e Invertebrati, e le secrezioni, come regola, dimostrarono di essere risorse difensive di straordinaria efficacia.

FIG. 1. Secretion oozing out of the lateral glandular openings of a millipede (*Narceus gordanus*) following stimulation by tapping with the small mallet shown.

FIG. 2. A tenebrionid beetle (*Diaperis maculata*) placed on a specially treated indicator paper that discolors in contact with the secretion. The beetle has been prodded, and is walking away leaving a trail on the paper.

FIG. 3. A coccinellid beetle (*Epilachna varivestris*) showing localized reflex bleeding in response to pinching of one of its legs with forceps.

FIG. 4. The spray of an earwig (*Forficula auricularia*) on special indicator paper. Earwigs aim their spray; the particular discharge shown here was produced in response to stimulation of the abdomen.

FIG. 5. A notodontid caterpillar (*Schizura leptinoides*) spraying on indicator paper in response to pinching with forceps.

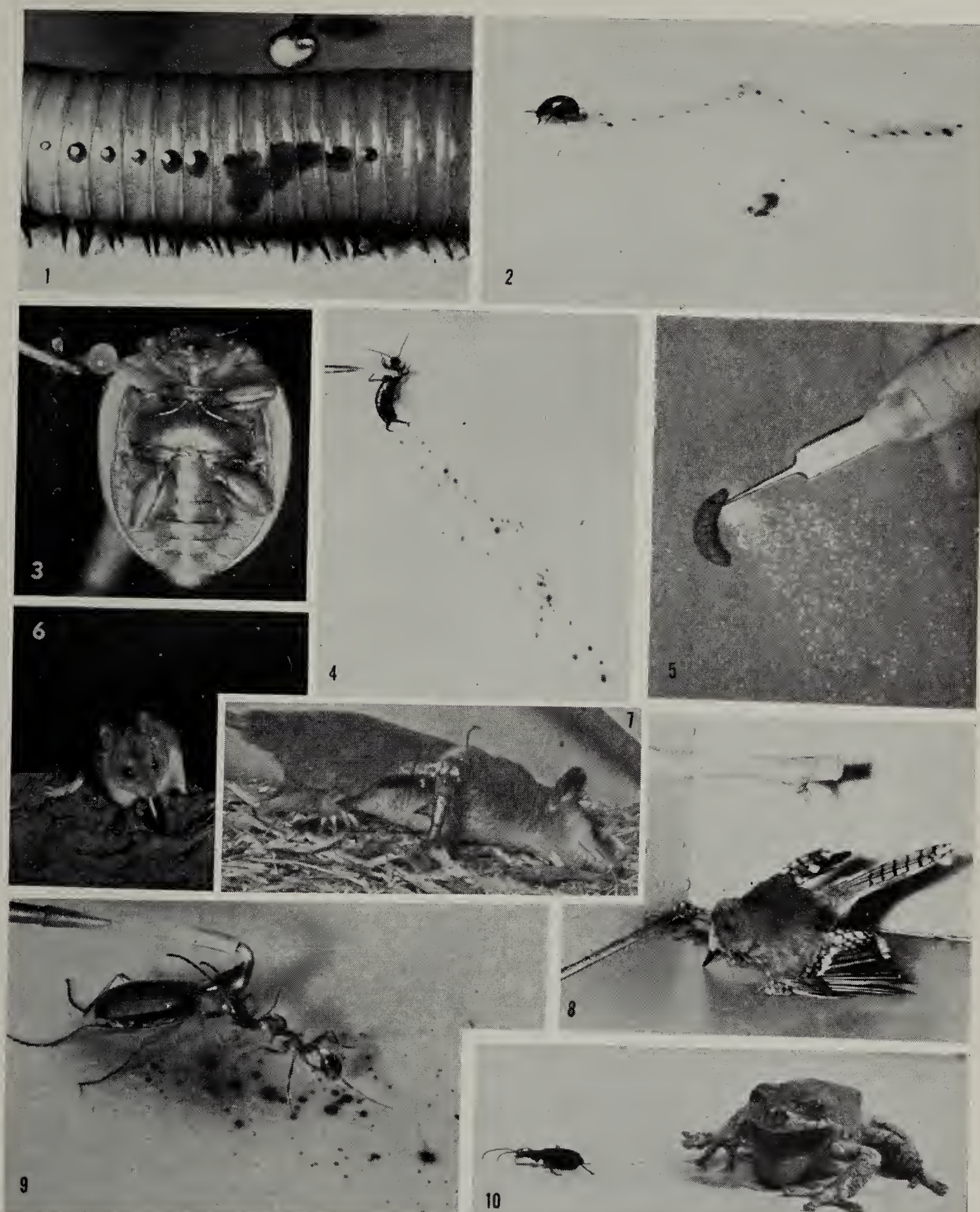
FIG. 6. A grasshopper mouse (*Onychomys torridus*) feeding on the tenebrionid beetle *Eleodes longicollis*. See text for details.

FIG. 7. An opossum scurrying with its face dragging in the substrate after having been sprayed during an attack on the walking stick *Anisomorpha buprestoides*. The walking stick is still held in the opossum's paw.

FIG. 8. A jay (*Cyanocitta cristata*) receiving the full impact of the spray of a walking stick (*Anisomorpha buprestoides*).

FIG. 9. A bombardier beetle (*Brachynus ballistarius*) ejecting an aimed discharge at an attacking ant (*Pogonomyrmex badius*).

FIG. 10. A small toad (*Hyla versicolor*) after rejecting a bombardier beetle (*Brachynus* sp.).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SCHILDKNECHT H. (*)

UNTERSUCHUNGSMETHODEN ZUR AUFKLÄRUNG CHEMISCHER ABWEHRSTOFFE VON INSEKTEN IX. MITTEILUNG ÜBER INSEKTENABWEHRSTOFFE (¹)

Die Analyse von Abwehrstoffen beginnt bereits mit der Isolierung, der unmittelbar die Anreicherung und die Trennung folgt, um mit einer Identifizierung oder Konstitutionsaufklärung zu enden. Da sich die meisten Insekten schlecht züchten lassen, steht das zu untersuchende Material oft nur in beschränkter Menge zur Verfügung, wodurch die klassischen Methoden zur Trennung und Aufklärung von Substanzgemischen nicht selten unzureichend werden. Überdies scheint es erstrebenswert, mit Substanzmengen der gleichen Grössenordnung zu arbeiten, wie sie im biologischen Elementarvorgang, z. B. bei einer Abwehrreaktion, tatsächlich auftreten. Im folgenden ist deshalb ausschliesslich von Mikromethoden die Rede.

Sind die zu untersuchenden Stoffe gasförmiger Natur, so bedarf es zur Analyse kleinster Mengen sehr moderner Hilfsmittel. Noch am ehesten kommt man zum Ziel, wenn man die der Reaktion zugrunde liegenden Muttersubstanzen vornimmt und aus ihnen das « Reaktionsgas » freisetzt, wie wir das bei der Aufklärung der Explosionschemie beim Bombardierkäfer (*Brachinus crepitans*) gezeigt haben (1). Durch eine massenspektrometrische Untersuchung bzw. durch eine quantitative Mikrogasanalyse nach Berg (2) kamen wir zu dem Ergebnis, dass das Explosionsgas bis zu 98 % aus Sauerstoff bestehen kann.

Meist sind aber die in Frage kommenden Stoffe dampfförmig, wenn nicht flüssig oder fest. Flüchtige Verbindungen isoliert man am vorteilhaftesten in selektiver Weise über die Gasphase durch Kondensation oder durch Lösen in einem vorgelegten Lösungsmittel. Eine einfache Extraktionsanordnung haben wir bereits beschrieben (3) und mit gutem Erfolg zur Gewinnung der flüchtigen Abwehrstoffe u. a. der Bombardierkäfer (1), vieler Tenebrioniden (3, 4, 5), Carabiden (6), Staphyliniden (7), Pentatomiden (7) und Myriapoden (6) ver-

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(¹) VIII. Mitteilung Ztsch. für Naturforschung, im Druck.

wendet. Man erhält auf diese Weise mehr oder minder stark verdünnte Lösungen oder Suspensionen, die für die eigentliche Analyse meist erst konzentriert werden müssen.

Elegant und schonend, vor allem ohne jegliche Verluste kann dies durch kontrolliertes Ausfrieren des Lösungsmittels geschehen. Beim « Normalen Erstarren », wie man ein solches Verfahren bezeichnet (8), wird die Lösung oder auch Suspension in einem passenden Reagenzglas langsam (5 bis 50 cm/h) vom Boden her eingefroren und dabei das jeweils über dem ausgefrorenen Lösungsmittel stehende Konzentrat stark gerührt (etwa 2000 bis 6000 U/min). Zum Schluss des Anreicherungsvorganges befinden sich fast immer die gesuchten Substanzen am Ende eines festen Lösungsmittelstabes und können dort in Substanz oder nach dem Auftauen einer geringen Lösungsmittelmenge, als Konzentrat isoliert werden. Das Verfahren hat den Vorteil, dass die chemische Natur der gesuchten Verbindungen nicht bekannt zu sein braucht und dass man selbst noch bei hochverdünnten Ausgangslösungen (1 µg/l) doch zu analytisch erfassbaren Konzentrationen kommt. Schon an dieser Stelle wird man oft eine Aussage über die Stoffklasse mit Hilfe spektroskopischer Methoden machen können, um dann die eigentliche Identifizierung in die Wege zu leiten.

Als die wichtigste Methode der Identifizierung muss hier die Papierchromatographie genannt werden, mit den reinen Verbindungen oder mit deren Derivaten.

Sie ist z. B. sehr gut geeignet für die Trennung und Analyse selbst eines komplexen Gemisches von Insektenchinonen, wo die bisher bekannten Nachweisreaktionen entweder nicht eindeutig genug, oder aber jeweils nur für eine sehr begrenzte Anzahl von Chinonen anwendbar waren.

Eine in allen Fällen durchführbare Reaktion ist die Umsetzung der Chinone mit 2,4-Dinitrophenylhydrazin (DNP), obgleich sich herausstellte, dass die DNP-Derivate der Hydroxychinone analytisch nicht besonders brauchbar sind. Diese Chinone können jedoch unter bestimmten Bedingungen als solche papierchromatographisch nachgewiesen werden, so dass sich nach Vorproben ein analytischer Trennungsgang für sehr viele p-Benzochinone durchführen lässt (5). Die Vorzüge sind dabei, dass man mit geringem Aufwand an Material schnell zu zuverlässigen Ergebnissen kommt. Selbst kleinste Chinonmengen, auch wenn sie im Gemisch vorliegen sollten, können noch bestimmt werden, da die Erfassungsgrenze für ein Chinon auf dem Chromatogramm noch unter einem $\mu g/cm^2$ liegt.

Nun sind aber viele Stoffe, die Insekten zu ihrem Schutz produzieren, fest, z. B. die Insektenwachse, und es bedarf nur einer sicheren Hand, um auch noch kleine Substanzmengen mechanisch zu isolieren und zu sammeln. Dagegen ist die Reinigung der fast immer unreinen Substanzen bzw. die Trennung von Gemischen in die einzelnen Komponenten nicht einfach, wenn man nur über geringe Mengen verfügt. Wir haben deswegen für kristallisierbare Stoffe ein Trenn- und Reinigungsverfahren ausgearbeitet, das immer dann eine verlustlose Bearbeitung des Material ermöglicht, wenn dieses ohne Zersetzung

schmilzt. Das Substanzgemisch wird beim Mikro-Zonenschmelzen (9) in einem kleinen Glasrohr oder-Boot eingetragen und an dicht nebeneinander liegenden Stellen mittels Heizschlingen in sehr schmale Bereiche aufgeschmolzen. Ein Zusammenschmelzen des Kristallbarrens wird durch Kühlschlingen verhindert, die zwischen den Heizschlingen angebracht sind. Die Stofftrennung erfolgt aber erst dann, wenn die Schmelzzonen den Schmelzling durchwandern, was man

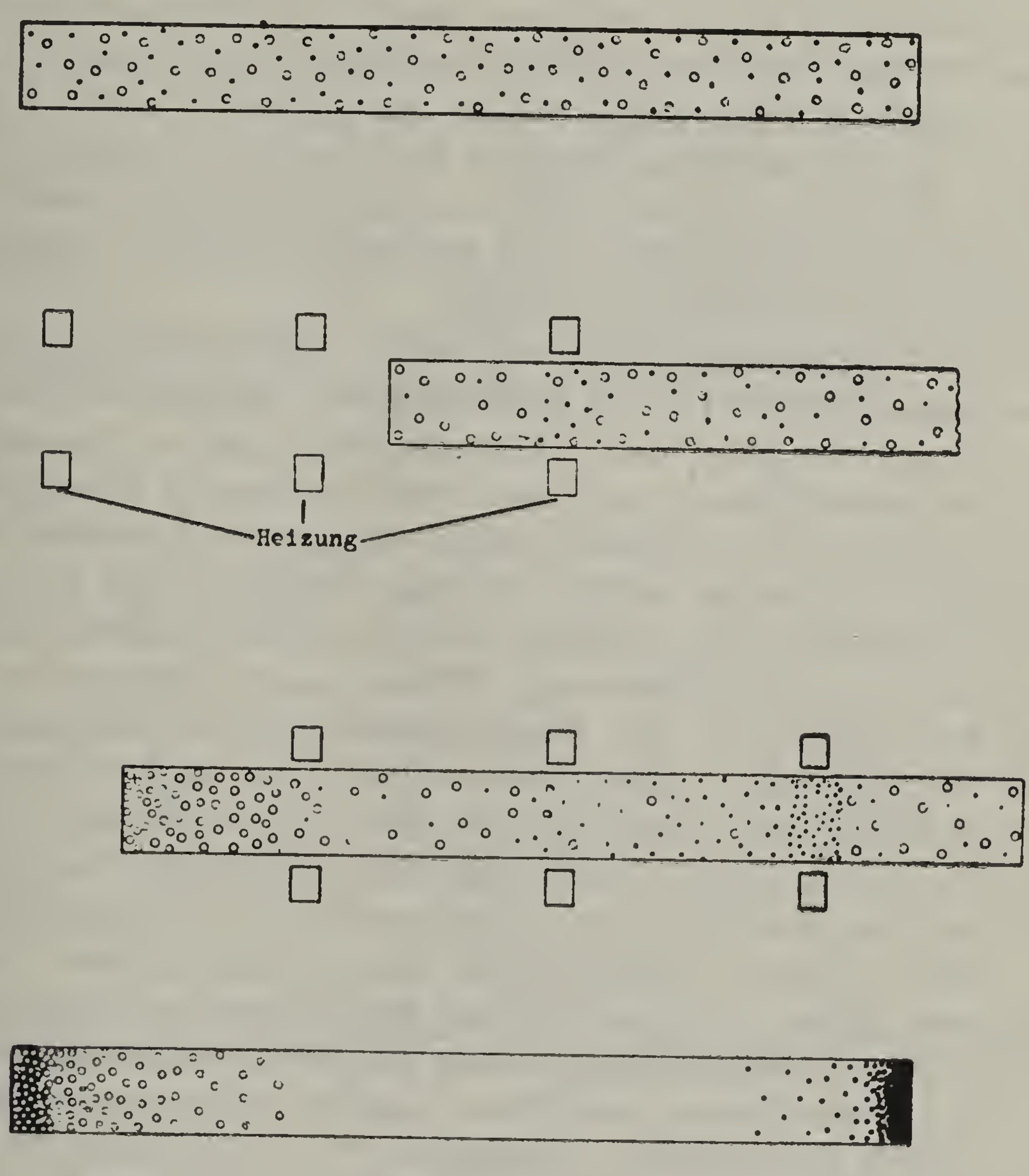

Abb. 1

Schematische Darstellung eines Zonenschmelzvorganges vor, während und nach dem Durchgang von 3 Schmelz-zonen.

erreicht, wenn man das Substanzrohr durch das Kühl-und Heizaggregat hindurchzieht. Der Trennvorgang soll kurz mit Hilfe der Abb. 1 erklärt werden.

Wir nehmen an, dass zunächst zwei Beimengungen, bezeichnet mit o und · in einem Grundmaterial so gleichmässig verteilt sind, dass eine feste Lösung

vorliegt. Die beiden Substanzen sollen verschiedenes Schmelzverhalten zeigen, etwa so, dass Substanz o den Schmelzpunkt des festen Lösungsmittels erhöht und die Substanz · ihn erniedrigt. Das hat aber zur Folge, dass sich die höher schmelzende Verbindung in den sich am Kristallisationsende abscheidenden Kristallen bevorzugt anreichert und die niedriger schmelzende mit der Schmelzzone an das Ende des Substanzbarrens wandert. Die Trennung wird also umsomehr fortschreiten, je mehr Schmelzzonen den Barren durchwandern, d. h. je mehr Öfen von diesem passiert werden. Sind viele Schmelzzonen — oft sind es 20 bis 50 und mehr — durch das Substanzgemisch gewandert, dann hat sich die eine Art Verbindung am rechten Ende, das zuletzt erstarrt, und die andere am linken Ende, dem zuerst erstarrenden Teil des Schmelzlings, angesammelt. Dazwischen liegt der Hauptteil der Substanz in hochreiner Form. Die zu trennenden Komponenten dürfen auch in vergleichbarer Menge nebeneinander vorliegen, aber nicht als eutektisches Gemisch.

Wir haben das Zonenschmelzen vor allem für die Untersuchung von Insektenwachsen angewandt, z. B. zur Trennung des Raupenwachses von Attacus edwardsii. Die Raupe ist über und über mit Wachspulver bepudert, das trotz mehrmaligem Umkristallisieren aus den verschiedensten Lösungsmitteln nicht gereinigt werden konnte. Der Schmelzpunkt lag immer konstant bei 82° C, was auf eine einheitliche Substanz schliessen liess. Lediglich das Infrarot-Spektrum zeigte eine Beimengung an, die mit den sonstigen Analysendaten nicht zu deuten war. Rein war das gut kristallisierende Wachs also nicht und wir versuchten deswegen die Aufspaltung in die Komponenten durch Zonenschmelzen. Das Ergebnis war überraschend: das Rohprodukt liess sich durch Ring-Zonenschmelzen (10) in Bestandteile zerlegen, die sich in ihrem Schmelzpunkt um 20° C unterschieden. Bei solchen wegen der Mischkristallbildung ihrer Komponenten schwer zu trennenden Wachsen war es interessant, zu untersuchen, ob die Isolierung einer oder mehrerer Bestandteile ohne Zuhilfenahme anderer Trenn- oder Reinigungsmethoden aus einer kleinen Menge, etwa einigen mg, möglich ist. Wir wählten für diese Untersuchungen das Wachs der Grasschildlaus Eriopeltis festucae (11). Die Wachsfasern sind hier insofern bemerkenswert, als sie eine mit Wachs umhüllte Proteinfaser darstellen. Wir sammelten ca. 3000 solcher Faserfilze mit einem Gesamtgewicht von 215 mg. Aus 100 mg entfernten wir durch Ausschmelzen und Abpressen das Wachs und erhielten aus dem mit Chloroform gewaschenen Rückstand 13,5 mg Proteinfasern. Ausgehend von 50 mg Rohwachs begannen wir die Fraktionierung durch Mikrozonenschmelzen. Die Schmelzlinge konnten zuerst noch nach den nicht fluoreszierenden (höher schmelzenden) und nach den fluoreszierenden (niedriger schmelzenden) Anteilen aufgetrennt werden. In der Folge der Fraktionierung aber wurde die Zuordnung der Fraktionen nach dem Schmelzpunkt vorgenommen. Die Identifizierung der aus dem Lauswachs isolierten reinen Substanz mit einem Schmp. von 84,3° geschah durch IR-Spektroskopie, wonach es sich wegen der charakteristischen Banden bei 729, 719, 1180 und 1190 cm^{-1} um einen langkettigen Wachsester handeln musste. Nach der genannten Analyse und durch einen Ver-

gleich mit einem synthetischen und zonengereinigten Wachs ist die Hauptkomponente des Wachses der Grasschildlaus der Hexakosansäure-hexakosylester.

Stand schon bei diesen Untersuchungen im Mittelpunkt der Konstitutionsaufklärung die IR-spektroskopische Analyse, so in noch ausgedehnterem Masse bei der Strukturaufklärung des harzartigen Abwehrstoffes der *Nasutitermes*-Soldaten. Als uns dankenswerter Weise Prof. R. Geigy (Basel) (12) gebeten hatte, die chemische Aufklärung des interessanten Abwehrstoffes zu übernehmen, hatten wir keinerlei Hinweis zu welcher chemischen Klasse der Stoff gehören könnte. Wenn wir heute aber trotzdem wenigstens eine Komponente als 24-Methylen-cholesterin identifizieren konnten, so verdanken wir es einer eingehenden IR-Analyse, die wir ohne jeglichen Substanzverlust mit wenigen mg des Abwehrstoffes ausgeführt haben, allerdings unter Zuhilfenahme vieler Vergleichssubstanzen ([1]), deren Spektren und Chromatogramme wir ebenfalls aufgenommen hatten. Der wichtigste Hinweis war von vornherein die auffallende Ähnlichkeit des IR-Spektrums mit dem Spektrum von Cholesterin, das durch seine relative Bandenarmut ausgezeichnet ist (s. Abb. 2).

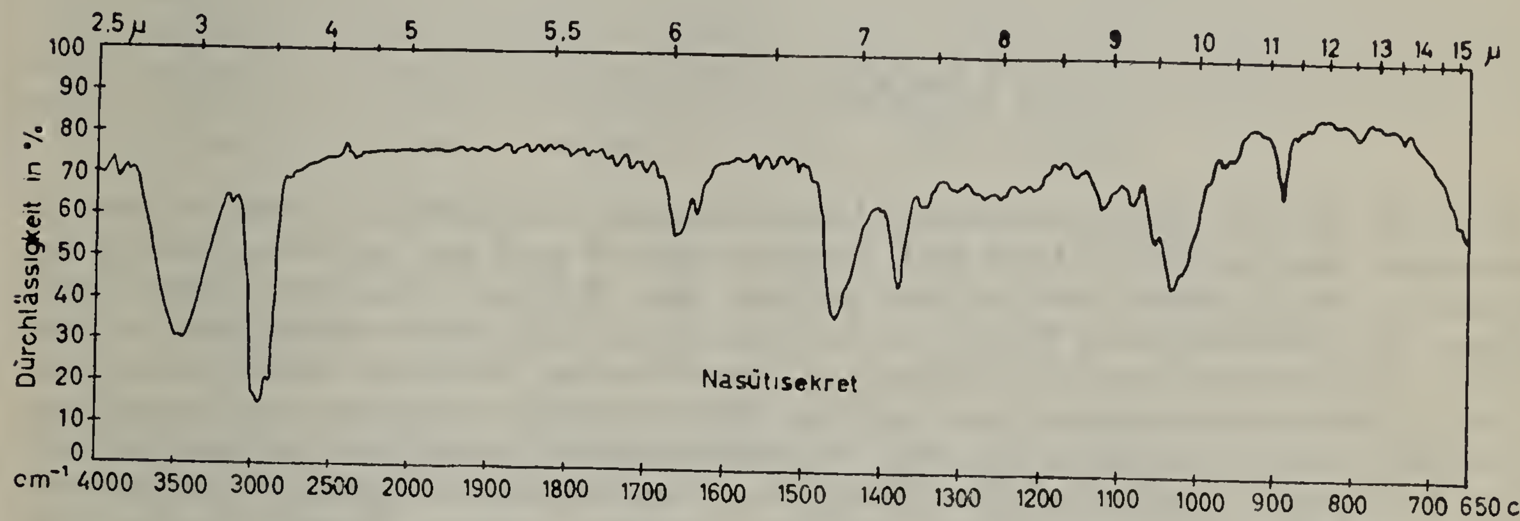

Abb. 2

IR-Absorptionsspektrum vom festen Sekret der *Nasutitermes*-Soldaten.

([1]) Den Herren Dr. M. Barbier und Dr. O. Schindler danken wir für die freundliche Überlassung von 24-Methylen-cholesterin.

Auch an dieser Stelle möchte ich den Herren Dr. K. Holoubek, Dr. H. Vetter, Dipl. Chem. K. H. Weis und Dipl. Chem. H. Schübel für ihre hervorragende Mitarbeit danken. Der Deutschen Forschungsgemeinschaft danken wir herzlich für die gewährte Unterstützung.

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ZUSAMMENFASSUNG

Es ist oft nur eine kleine Anzahl von Individuen eines Gliederfüsslers, mit der man im Laboratorium experimentieren kann und deswegen sind es auch nur spärliche Mengen des unbekannten Abwehrstoffes über die man verfügen darf. Will man dann noch seine Untersuchungen in wünschenswerter Weise z. B. auf alle Arten einer Gattung ausdehnen, so muss man nicht selten auf die klassische Methode der Anreicherung, Trennung und Identifizierung von Substanzgemischen verzichten und moderne Mikro-Verfahren anwenden bzw. entwickeln. Gase können sicher und elegant mit dem Massenspektrometer identifiziert werden, wie sich bei der Analyse des Bombardierkäfers gezeigt hat. Dampfförmige Verbindungen lassen sich mit einer einfachen Absaugvorrichtung isolieren, mit dem grossen Vorteil, dass nur die wesentlichen, zur Abwehr ausgestossenen Substanzen erfasst werden. Allerdings sind die so gewonnenen Lösungen manchmal noch sehr verdünnt, können aber durch «normales Erstarren» oder bei Mischkristallbildung durch «Eis-Zonenschmelzen» konzentriert werden. Es ist uns gelungen, auf diese Weise unter anderem nicht mehr nachweisbare Mengen von Hydrochinon und Insektenchinone weit über die Erfassungsgrenze hinaus anzureichern. Auch aus festen Lösungen lassen sich Substanzen durch Zonenschmelzen isolieren und, was. u. U. wichtiger ist, Substanzgemische trennen, vor allem Insektenwachse und fettartige Gemische. Nach der erfolgten Anreicherung bzw. Trennung ist für die Ermittlung der Stoffklasse Ultraviolettspektroskopie unentbehrlich, sowie für eine weitere Auftrennung chemisch verwandter Bestandteile mit zusätzlicher und nicht selten endgültiger Identifizierung chromatographische Verfahren. Die Struktur kompliziert gebauter Stoffe kann auch mit kleinsten Mengen durch eine eingehende Konstitutionsanalyse mittels Infrarotspektren ermittelt werden, wie sie z. B. im Mittelpunkt unserer Untersuchungen über den Abwehrstoff von *Nasutitermes* stand. Danach ist das harzartige Sekret ein Gemisch, dessen Hauptmenge ein zweifach ungesättigtes Oxy-Sterin ist.

RIASSUNTO

Metodi di ricerca per lo studio dei mezzi chimici di difesa negli Insetti.

IX. Comunicazione sui mezzi di difesa degli Insetti.

Spesso in laboratorio si può sperimentare soltanto con un piccolo numero di individui di una determinata specie di Artropodo, e quindi sono disponibili solo quantità ridotte della sostanza di difesa ignota. Se poi si vogliono estendere le ricerche per es. a tutte le specie d'un certo genere, bisogna non raramente rinunciare al metodo classico dell'arricchimento, separazione e identificazione dei miscugli e utilizzare o sviluppare moderni microprocedimenti. I gas possono essere identificati con sicurezza ed eleganza mediante lo spettrometro di massa, come si è dimostrato nella analisi del Coleottero bombardiere (*Brachinus*). Composti allo stato di vapore si isolano con una semplice apparecchiatura di aspirazione col grande vantaggio di catturare soltanto le sostanze essenziali emesse per la difesa. In ogni modo le soluzioni così ottenute sono talora ancora troppo diluite, ma possono venir concentrate per solidificazione normale o con formazione di cristalli misti mediante fusione per zone del ghiaccio. Siamo riusciti tra l'altro ad arricchire in questo modo quantità non più individuabili di idrochinone e chinoni di Insetti ben oltre il limite di riscontrabilità. Mediante fusione per zone si possono isolare sostanze anche da soluzioni solide e, cosa importante in determinate condizioni, da miscugli, soprattutto cere di Insetti e miscugli grassi. Dopo l'arricchimento e la separazione, per classificare la sostanza è indispensabile la spettroscopia all'ultravioletto, come lo sono i procedimenti cromatografici per una più precisa distinzione di composti chimicamente affini e per una ulteriore e non raramente definitiva identificazione. La struttura di sostanze complesse si può studiare su quantità anche molto piccole mediante una profonda analisi costitutiva con spettri infrarossi, come fu fatto per es. durante le nostre ricerche sulla sostanza di difesa di *Nasutitermes*. In base a queste indagini, il secreto resinoso è un miscuglio il cui componente principale è un'ossisterina doppiamente insatura.

PAVAN M. (*)

ESTRAZIONE E PURIFICAZIONE DI ALCUNI COMPONENTI DELLE SECREZIONI DIFENSIVE DI ARTROPODI

1. INTRODUZIONE

Lo studio chimico e biologico delle secrezioni degli Artropodi ci ha fornito, specialmente negli ultimi anni, dati di grande interesse ed appare perciò probabile che questo tipo di ricerche subisca futuri notevoli sviluppi. Anche limitandosi al campo di studio delle secrezioni difensive degli Insetti, l'argomento si presenta con ricche prospettive: è stato infatti recentemente calcolato che mentre furono studiati (fino all'isolamento e riconoscimento chimico di sostanze componenti il veleno) circa 60 specie di Insetti, si può calcolare che esistano almeno 50.000 specie velenose (11, 12).

In questi studi uno degli aspetti più delicati è dato dal divario fra l'esigenza di abbondante materia prima, fonte delle sostanze da isolare e studiare, e la generale difficoltà di procurarla; altro aspetto che presenta difficoltà è quello di ottenere l'isolamento dei costituenti dei veleni. Perciò ho ritenuto non privo di interesse pratico e scientifico fornire alcuni esempi di sistemi di raccolta della materia prima (Artropodi) e di isolamento delle sostanze interessanti.

Questo breve lavoro è limitato ad alcuni fra i casi che hanno formato oggetto delle indagini mie e con vari Collaboratori (v. bibliografia). Una raccolta di dati più completa è auspicabile: essa sarà molto utile in considerazione del fondamentale significato pratico che può avere per lo sviluppo ulteriore di questo interessante campo di lavoro chimico-biologico.

Per ogni specie di animale considerata, riporto alcuni dati molto riassuntivi sul genere di vita e sull'ambiente in cui può venire raccolta e cenni sui sistemi di raccolta. Successivamente per ogni specie riferisco i sistemi impiegati per ottenere il veleno e le prime tappe seguite per la purificazione. In alcuni casi (ad s. *Tapinoma*, *Blaps*, *Schizophyllum*) rimando però ai lavori originali per non dover scendere in questa nota a particolari procedimenti chimici e fisici troppo specializzati.

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Nell'estrazione e purificazione dei costituenti dei veleni hanno avuto importanza fondamentale le reazioni biologiche che permisero di individuare la presenza e il grado di concentrazione dei prodotti. Nei casi trattati in questa nota i tests biologici furono impostati sulla reazione di intossicazione di varie specie di insetti trattati con i prodotti ricavati. Altre reazioni di controllo su animali e su vegetali furono di aiuto nelle fasi di lavoro (ad es. reazione di repellenza, attività antibatterica, antimitotica, fitoinibente, reazione olfattiva umana, ecc.).

2. RACCOLTA E PREPARAZIONE DEGLI ANIMALI

HYMENOPTERA FORMICIDAE. *Iridomyrmex humilis* Mayr, *Tapinoma nigerrimum* Nyl., *Liometopum microcephalum* Panz., *Myrmicaria natalensis* Fred.

Queste specie vivono nel terreno. Le operaie formano compatte colonne sul terreno, sulle piante, sui muri degli edifici.

Materiale per la raccolta: 1) un recipiente cilindrico di metallo leggero, diametro cm 25, altezza cm 40, parete interna lucida; sul fondo uno strato *compatto* di 3 cm di fibra di cotone con sovrapposto diaframma di rete metallica molto fine (maglie di 1 mm) saldata alla parete del recipiente; 2) fili di ferro diametro 3 mm, lunghi 30-40 cm.

Si piega a S un'estremità dei fili di ferro applicandovi un pezzo di carne o pesce e si innestano sul terreno per l'estremità diritta nei pressi delle colonne di formiche.

Le formiche salgono in massa sull'alimento. Periodicamente si prelevano da terra i fili di ferro e si scuotono nel recipiente metallico sul cui fondo, al di sotto della rete metallica, vi è il cotone compresso previamente imbevuto di etere etilico + cloroformio. Le formiche che cadono nel recipiente entrano subito in narcosi a causa dei vapori della miscela etere-cloroformio.

Si possono così raccoglire di seguito le formiche di molte decine di fili di ferro. Alla fine del giro di raccolta le formiche in narcosi vengono rapidamente liberate da eventuali frammenti di sostanze estranee; così pulite sono pronte per le operazioni di ulteriore conservazione (ad esempio in congelatore a — 20° C) o per l'immediata estrazione chimica.

Lasius (*Dendrolasius*) *fuliginosus* Latr., *Liometopum microcephalum* Panz.

Vivono nei tronchi di piante d'alto fusto ove scavano vani anche di cm 50 × 100 per l'alloggiamento dei nidi; riempiono questi vani con fitte impalcature di « cartone » fabbricato con fibre di legno agglutinato con le secrezioni delle glandole mandibolari. Per la cattura si individua nell'estate il nido (colonne di operaie); nell'inverno, quando la popolazione è raccolta in letargo nel nido, si allarga il foro d'ingresso del nido e frugandovi con un legno si sgretola il cartone che si fa uscire e cadere in recipienti contenenti cotone imbevuto di anestetico. In laboratorio si separano le operaie dal materiale estraneo.

Liometopum può essere però raccolto anche in estate col sistema descritto per *Iridomyrmex*.

Un grande nido di queste specie può dare anche oltre 500 g di operaie. Si possono anche trovare colonie con 5 nidi per ettaro.

Coleoptera Tenebrionidae. *Blaps mucronata* Latr., *Blaps gibba* L.

Gli adulti si raccolgono in natura nelle cantine umide, nei depositi di legname. Alla sera e di notte escono dai nascondigli. Vivono a lungo in allevamento, in recipienti con terra, pezzi di legno, materiali di scarto (vegetali, pane) di cui si alimentano. Alla fine del ciclo le larve si trasformano in ninfa alla superficie del terreno sotto materiali di riparo. Gli adulti possono fornire numerose cariche di veleno.

Blaps requieni Sol.

Gli adulti, deserticoli, si raccolgono in grande quantità sotto le pietre in Africa settentrionale. Escono dai ripari alla sera e nella notte. Gli adulti vivono a lungo in terrari e si possono ricavare da essi numerose cariche di veleno.

Coleoptera Chrysomelidae. *Melasoma populi* L.

Le larve vivono soprattutto sulle foglie dei pioppi. Vengono raccolte staccando le foglie. Possono essere tenute a lungo vive in laboratorio con alimentazione di foglie di pioppo fresche. Gli adulti depongono le uova facilmente e le larve possono essere allevate. Ogni larva può dare ripetute scariche di veleno.

Lepidoptera Cossidae. *Cossus cossus* L.

Le grosse larve vivono nel legno di varie piante (pioppo, salice, ecc.) e si trovano spaccando i tronchi. Per la raccolta si possono distribuire scatole metalliche agli operai addetti all'abbattimento e alla spaccatura delle piante e nelle segherie. Le larve che vengono alla luce possono essere conservate vive anche per vari giorni nella segatura umida.

Myriopoda. *Archiulus* (*Schizophyllum*) *sabulosus* L.

E' specie che vive nel terreno sotto i sassi o fra i detriti vegetali. Possono vivere a lungo in recipienti con terra, humus, muschio. Ogni individuo può fornire ripetute cariche di veleno.

3. ESTRAZIONE E PURIFICAZIONE DEI VELENI

Iridomyrmex humilis: iridomirmecina.

Si separa l'estratto etereo di operaie di *Iridomyrmex humilis*. Si maciullano i corpi e si pressano; il liquido ricavato ed i corpi si esauriscono con etere, a freddo o in Soxhlet. Tutti gli estratti eterei vengono riuniti e l'etere viene distillato: si ottiene così un residuo oleoso dal quale per sublimazione a 60-100° C si ottiene l'iridomirmecina cristallina.

Il residuo oleoso originale, dopo riposo prolungato al fresco con ampia superficie esposta all'aria può dare luogo lentamente a cristallizzazione spontanea di iridomirmecina impura. Il prodotto puro si ottiene per sublimazione dell'iridomirmecina impura miscelata con polvere di carbone animale.

Tapinoma nigerrimum: metileptenone, propilisobutilchetone, iridodial.

L'estratto etereo dei corpi viene privato del solvente per distillazione e si ottiene un residuo oleoso intensamente profumato. Questo viene sottoposto a distillazioni frazionate sotto vuoto raccogliendo le frazioni che passano fra 50° e 110° C ed il residuo oleoso finale: nelle varie frazioni sono variamente ripartiti i costituenti del veleno; questi si ottengono allo stato puro con procedimenti chimici e fisici che abbiamo descritto (21).

Lasius (Dendrolasius) fuliginosus: dendrolasina.

Per estrarre dal corpo totale il principio profumato, si sottopongono i corpi freschi ad estrazione in etere di petrolio bassobollente. Indi si sottopongono i corpi a pressatura con sabbia di quarzo a 300 atmosfere, si separa la frazione oleosa natante; si lava il residuo acquoso e i corpi con nuovo etere di petrolio: riunite le frazioni eteresolubili, si distilla a bassa temperatura fino a completo allontanamento del solvente. Il residuo oleoso intensamente profumato si distilla in corrente di vapore. Si esauriscono le acque di condensazione mediante etere di petrolio bassobollente. Si allontana l'etere di petrolio per distillazione. Si ottiene una frazione oleosa, gialla, fluida, contenente circa il 75 % di dendrolasina. Per successiva distillazione si ottiene la dendrolasina pura, liquida incolora, intensamente profumata.

Myrmicaria natalensis: *d* e *l*-limonene.

Si possono seguire due sistemi:

1) I corpi di operaie vengono posti in etere etilico; l'estratto etereo viene distillato per l'eliminazione del solvente; il residuo oleoso viene distillato in corrente di vapore fino a completo passaggio delle sostanze veicolabili; le acque di condensazione vengono esaurite con etere etilico.

2) I corpi delle operaie uccise con vapori di etere etilico vengono cosparsi con NaCl in polvere fine (20 % del peso dei corpi) che permette una lunga conservazione anche in climi equatoriali. Successivamente i corpi vengono macinati a secco in mulino a palle e la pasta viene distillata in corrente di vapore. Le acque di condensazione vengono riprese in etere etilico.

In ambedue i sistemi l'estratto etereo viene lavato con soluzione di $NaHCO_3$ per eliminare le sostanze acide e successivamente seccato su Na_2SO_4 anidro. Dopo distillazione del solvente si ottiene un residuo liquido che distillato a pressione ordinaria passa fra 100° e 180° C fornendo un liquido incoloro con forte odore terpenico. A 170° - 175° C passano soprattutto *d* e *l*-limonene, le sostanze che conferiscono il caratteristico odore al veleno di questa specie ([1]).

Melasoma populi: aldeide salicilica.

Il secreto delle larve si ottiene con un'operazione di « mungitura »: a tale scopo si prendono le larve vive con una pinza lungo la linea sagittale nella regione dorsale del torace; sotto questa eccitazione le larve emettono degli orifizi di ogni serbatoio una gocciola di secreto che viene aspirato e conservato in una microsiringa. Tale secreto tende a frazionarsi in due porzioni; mediante soggiorno in frigorifero e successiva centrifugazione a 6000 giri al minuto a 0° C, si fraziona in due parti: una frazione natante inodora (priva di sostanze biologicamente attive) ed una frazione di fondo, avente l'odore tipico del secreto. Questa frazione è lavata con H_2O distillata, riottenuta per centrifugazione refrigerata (+ 2° C) e separata dall'acqua di lavaggio. La frazione così ottenuta ha fluorescenza marron-opaca alla luce di Wood, il tipico odore del secreto della larva e presenta le attività biologiche del materiale di origine ma concentrate. Essa è costituita da aldeïde salicilica.

Blaps gibba, *B. mucronata*, *B. requieni*: vari benzo-chinoni.

Per ottenere il veleno si procede come descritto per il Miriapode *Archiulus* (*Schizophyllum*) *sabulosus*. I vari benzo-chinoni componenti dei veleni di queste specie sono separabili con sistemi chimici e fisici come descritto in 18.

Cossus cossus: cossina

Le larve, che a maturazione raggiungono anche 8-9 cm di lunghezza, vengono fissate in tensione e sotto narcosi da CO_2 od etere, su tavolette mediante spilli alle due estremità del corpo. Si incide il dorso lungo la linea mediana fino al capo e si mettono a nudo i due grandi serbatoi contenenti il secreto (diametro 2-4 mm, lunghezza 20-30 mm). Con pinza si afferra il canale deferente (presso il capo), si strappa e si solleva il serbatoio, si adagia su carta bibula per asciugarlo dall'emolinfa e si incide con ago facendo colare in provetta il secreto trasparente e leggermente giallo; il secreto può essere anche aspirato con siringa pungendo il serbatoio.

Il secreto puro così raccolto, intensamente odoroso, è composto dai tre costituenti principali cossina A, cossina B, cossina C, la cui costituzione è per ora solo parzialmente chiarita. I tre costituenti sono difficilmente separabili per distillazione frazionata; si ottiene la separazione con cromatografia in fase di vapore. Per ora col nome generico di cossina abbiamo indicato la miscela naturale dei tre componenti che risultano essere acetati di alcoli C_{14}, primari a catena lineare, insaturi, con il componente, o i componenti più abbondanti, forniti di due doppi legami separati da 8 atomi di carbonio.

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(1) Dai lavaggi alcalini con soluzione di $NaHCO_3$, per acidificazione con H_2SO_4 ed estrazione con etere sono stati ottenuti gli acidi grassi: acido acetico, ac. propionico, ac. isobutirrico, ac. isovalerianico. Per ora non è possibile chiarire se questi acidi grassi siano prodotti dall'organo del veleno, o da altri organi.

Archiulus (Schizophyllum) sabulosus: 2-metil-1,4-benzochinone; 2-metil-3-metossi-1,4-benzochinone.

In provetta contenente pochi cc di acqua distillata si pongono alcuni individui che vengono leggermente stimolati con una bacchetta di vetro in modo da obbligarli ad emettere il veleno giallo. Successivamente gli individui vengono trasferiti in altra provetta e lavati in acqua. Si ripetono le operazioni con altri individui fino a ottenere una prima acqua intensamente colorata di giallo-bruno. Questa viene saturata con NaCl puro ed estratta a freddo per scuotimento con etere etilico. Dopo breve riposo, si separa la parte eterea colorata. Si filtra la parte acquosa emulsionata per ricavare la residua porzione eterea. Le frazioni eteree contengono i chinoni del veleno che vengono separati con procedimenti vari descritti nel lavoro su questa specie (18).

I Miriapodi riposti in allevamento e ben nutriti (pane, piccole quantità di carne) servono ripetutamente per ulteriori produzioni di veleno. (Analoghe operazioni si possono fare con i Coleotteri del gen. *Blaps* produttori di chinoni).

CONCLUSIONI

Abbiamo visto sommariamente alcuni aspetti dello studio dei veleni di Artropodi, cioè la ricerca e l'approvvigionamento dei quantitativi necessari di materia prima e le fasi di lavorazione per ottenere i costituenti dei veleni di determinate specie. I dati esposti sono da considerare come schemi di lavoro la cui validità potrà in parte anche essere estesa ad altri campi di lavoro analoghi. Con i sistemi descritti abbiamo studiato varie specie di Artropodi ricavando la conoscenza di diverse sostanze costituenti il loro veleno, alcune delle quali nuove nella letteratura chimica (iridomirmecina, dendrolasina, cossina A, B, C) o rappresentanti di categorie chimiche non note per il regno animale (ad esempio i furani). Ciò ha portato a conoscere più a fondo la composizione dei veleni e delle loro proprietà biologiche aprendo anche proficui sviluppi di ricerche come risulta dalla letteratura chimica e biologica che si è sviluppata in buona parte come conseguenza di queste ricerche. Ciò autorizza quindi ad auspicare ulteriori sviluppi e perciò si può ritenere che una più ampia raccolta di dati sulle fasi di lavoro di cui abbiamo esposto alcuni aspetti in questa nota, possa rivestire un notevole significato per le future ricerche dei biologi e dei chimici.

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RIASSUNTO

Sono brevemente descritti i sistemi usati per ottenere grandi quantità delle specie di Artropodi sottoelencate, ed i metodi di estrazione e purificazione delle loro secrezioni per il successivo studio chimico dei principi attivi. Le specie di Artropodi considerate sono:

Formicidae: *Lasius (Dendrolasius) fuliginosus* Latr., *Myrmicaria natalensis* Fred., *Liometopum microcephalum* Panz., *Tapinoma nigerrimum* Nyl., *Iridomyrmex humilis* Mayr.

Coleoptera: *Blaps mucronata* Latr., *Blaps requieni* Sol., *Blaps gibba* L.; *Melasoma populi* L. (larve).

Lepidoptera: *Cossus cossus* L. (larve).

Myriopoda: *Schizophyllum sabulosus* L.

Le sostanze isolate sono: dendrolasina, d, l-limonene, iridomirmecina, iridodial, metil-eptenone, propil-isobutil-chetone, 1,4-chinone (p-benzochinone), 2-metil-1,4 chinone, aldeide salicilica, ecc.

SUMMARY

Extraction and purification of some components of the Arthropods' defensive secretions.

The methods used to obtain large quantities of the under-mentioned Arthropod species and the methods of extraction and purification of their secretions for subsequent chemical study, are briefly described. The Arthropod species are:

Formicidae: *Lasius (Dendrolasius) fuliginosus* Latr., *Myrmicaria natalensis* Fred., *Liometopum microcephalum* Panz., *Tapinoma nigerrimum* Nyl., *Iridomyrmex humilis* Mayr.

Coleoptera: *Blaps mucronata* Latr., *Blaps requieni* Sol., *Blaps gibba* L.; *Melasoma populi* L. (larvae).

Lepidoptera: *Cossus cossus* L. (larvae).

Myriopoda: *Schizophyllum sabulosus* L.

The substances in question are the following: dendrolasin, d, l-limonene, iridomyrmecin, iridodial, methyl-heptenone, propyl-isobutyl-ketone, 1,4-quinone (p-benzoquinone), 2-methyl-1,4 quinone, salicyl aldehyde, ecc.

CAVILL G. W. K., HINTERBERGER H. (*)

DOLICHODIAL AND RELATED COMPOUNDS [1]

INTRODUCTION

Previous investigations of Dolichoderine ant extractives have resulted in the isolation of a novel group of compounds: the cyclopentanoid monoterpenes. Thus iridomyrmecin (I) has been isolated from the Argentine ant, *Iridomyrmex humilis* (Mayr.) (1, 2), isoiridomyrmecin (iridolactone) (II) from *I. nitidus* (Mayr.) (3), and iridodial (III) from *I. detectus* (F. Sm.) and *I. conifer* (Forel) (4), and from *Tapinoma nigerrimum* (Nyl.) (5). Methylheptenone (5, 6) (IV) and propyl isobutyl ketone (5) (V) have been identified in association with iridodial.

More recently, a new terpenoid extractive, dolichodial, has been isolated from several *Dolichoderus* and *Iridomyrmex* species. *D.* (*Acanthoclinea*) *Clarki* (Wheeler), which is a more accessible species near Sydney, has been the source of dolichodial for the present chemical studies.

ISOLATION AND CHARACTERIZATION OF THE EXTRACTIVES FROM *DOLICHODERUS* (*ACANTHOCLINEA*) *CLARKI*

Spectroscopic and chromatographic techniques have been used, in addition to the classical methods of chemical degradation, in the course of the isolation, characterization, and structure determination of these ant extractives. Currently, a light petroleum extraction of *D. (Acanthoclinea) Clarki* has given a crude product, from which the major constituent, dolichodial, is obtained as a colourless, lachrymatory oil, b.p. 96°/2 mm, $[\alpha]_D^{22,5^0}$ -26°. The distillate also contains minor aldehydic and ketonic components.

Characterization of dolichodial, and of the minor constituents, is achieved by reaction of the distillate with 2,4-dinitrophenylhydrazine. This reagent is very widely employed for the characterization of carbonyl compounds. It nor-

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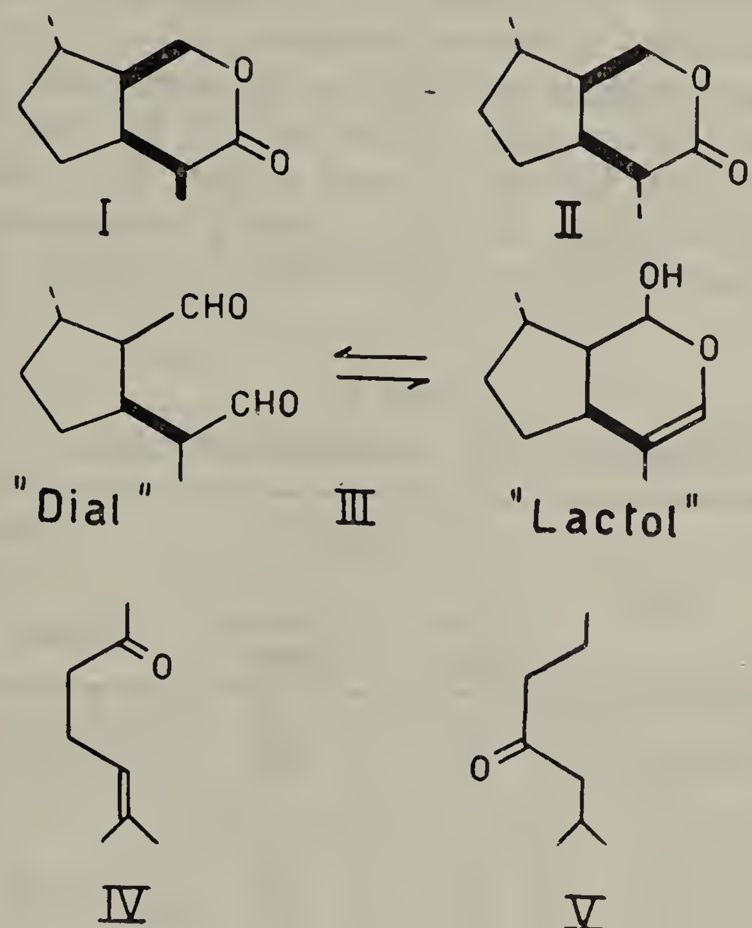
(1) A detailed account of the structure and reactions of dolichodial is to be submitted for publication in the Australian Journal of Chemistry.

mally yields crystalline derivatives, which are readily isolated, and thus permit of the characterization of even trace quantities of carbonyl extractives, in good yield. Moreover, the colour of these derivatives assists in their separation and identification by paper chromatography, or on a larger scale, by column chromatography. In the latter case, elemental analysis is then possible. Of the many



derivatives available for the characterization of aldehydes and ketones, the 2,4-dinitrophenylhydrazones still appear the most suitable (cf. 7), even though they are not readily hydrolysed to give the parent aldehyde or ketone.

Dolichodial gives two *bis*-derivatives, B and C, on treatment with 2,4-dinitrophenylhydrazine sulphate solution. An accurate analysis of the parent compound has not been possible, but on the basis of a $C_{22}H_{22}N_8O_8$ formulation for the above derivatives, it has been assigned a $C_{10}H_{14}O_2$ structure. Derivative B, m.p. 177°, which is yellow, is converted into the red compound, derivative C, m.p. 242°, by the action of mineral acid. It is probable that these derivatives are stereoisomers.

The distillate from ants collected during the spring and early summer months also contains a small proportion of a second carbonyl compound, isolated as an orange 2,4-dinitrophenylhydrazone, derivative A, m.p. 130-131°. This derivative, $C_{16}H_{18}N_4O_5$, is readily separated from the *bis*-derivatives of dolichodial by chromatography on alumina. The fat fraction which remains after

distillation has not yet been investigated. The forerunnings from the distillation of dolichodial contain a trace of a C_7 carbonyl compound, isolated as its orange 2,4-dinitrophenylhydrazone, derivative E, $C_{13}H_{18}N_4O_4$, m.p. 57°.

Ants collected during the winter months gave a smaller yield of dolichodial, but in addition, they contained an oxo-acid, isolated from the crude extract with sodium hydrogen carbonate solution. It is characterized as a yellow 2,4-dinitrophenylhydrazone, derivative D, $C_{16}H_{18}N_4O_6$, m.p. 170°, which is soluble in sodium hydrogen carbonate solution.

Table 1 gives data for these derivatives, in particular, the Rf values have been determined, using the methods of Burton (8), for the direct phase, and of Seligman and Edwards (9), for the reverse phase. The various *bis*-derivatives with 2,4-dinitrophenylhydrazine are more readily characterized by their infra-red spectra («Nujol» mulls) in the 700-1000 cm^{-1} region, than by paper chromatography.

TABLE 1.

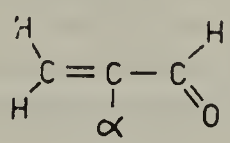
Extractives of *D.* (*Acanthoclinea*) *Clarki.*

2,4-Dinitrophenyl-hydrazone	M. P.	Rf values (*)		Parent compound	
		Direct phase	Reverse phase	Formula	Functional group/s
Derivative A	130-131° C	0.0	0.55	$C_{10}H_{14}O_2$	Carbonyl, and possibly an alcohol.
Derivative B (1) Derivative C (1)	177° 242°	0.0 0.0	0.15 0.0	$C_{10}H_{14}O_2$	Dialdehyde.
Derivative D	170°	0.05	0.95	$C_{10}H_{14}O_3$	Carbonyl and carboxyl.
Derivative E	57°	0.70	0.35	$C_7H_{14}O$	Ketone.
(±) 4-Methylhexan-2-one (IX)	41°	0.70	0.35	$C_7H_{14}O$	Ketone.
2-Methylhept-2-en-6-one (IV)	86°	0.60	0.25	$C_8H_{14}O$	Ketone.
Iridodial (III) (1)	228°	0.0	0.0	$C_{10}H_{16}O_2$	Dialdehyde.

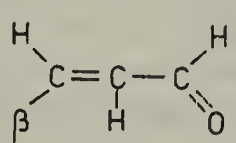
(*) Rf values, as reported, are ± 0.05.
(1) *Bis*-derivatives.

STRUCTURE OF DOLICHODIAL

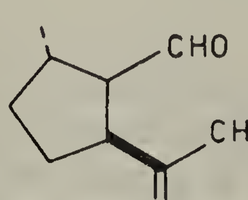
Dolichodial has an absorption in the ultraviolet region at 223 mμ (ε, 6,950 in water), characteristic of an α-, or a β-monosubstituted, α, β-unsaturated aldehyde (type VI or VII). Comparably, crotonaldehyde has λ max 223 mμ (in water). The infrared spectrum of dolichodial shows a strong band at 1725 cm^{-1}, and a medium band at 2720 cm^{-1}, attributable to the carbonyl group of an aliphatic aldehyde (10). Further, a strong band at 1690 cm^{-1}, and a medium band at 1633 cm^{-1}, are characteristic of the carbonyl group, and the carbon-



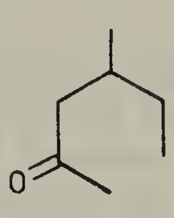
VI



VII



VIII



IX

carbon double bond, of an α, β-unsaturated aldehyde (cf. α-methylacraldehyde, which has absorption at 1695 cm^{-1} and 1639 cm^{-1}) (11). Thus dolichodial may contain an isolated aldehyde group, and an α, β-unsaturated aldehyde group.

Hydrogenation of dolichodial in the presence of a palladium catalyst, results in the uptake of one molecular proportion of hydrogen, and the band at 223 mμ is reduced in intensity to 15 % of that of the control. The reaction product gives an immediate precipitate with 2,4-dinitrophenylhydrazine, whence chromatography on alumina yields two products: a red derivative, m.p. 217°, and the major component, a yellow derivative, m.p. 228°. The latter derivative is identical with iridodial *bis*-2,4-dinitrophenylhydrazone. Further, it has been established that the derivative, m.p. 217°, is a stereoisomer of the derivative, m.p. 228°.

This transformation of dolichodial into iridodial shows that these compounds have the same cyclopentanoid monoterpene skeleton, and as dolichodial has an absorption at 223 mμ, attributable to a monosubstituted acraldehyde, it

must be represented by structure (VIII). Finally, the isolation of formaldehyde (in 30 % yield) from an ozonolysis of dolichodial, confirms this α-acraldehyde formulation (i.e. VIII). Dolichodial is related sterically to iridodial.

STRUCTURE OF DERIVATIVE E

Derivative E, m.p. 57°, behaves chromatographically as a C_7 methyl ketone (see Table 1). On the biogenetic assumption that the cyclopentanoid monoterpenes and related compounds are derived from citral, or citronellal, the parent compound of derivative E, which is optically active, could have structure (IX). From a comparison of the 2,4-dinitrophenylhydrazone of racemic 4-methylhexan-2-one, m.p. 41°, which has been synthesised, with derivative E, m.p. 56-57°, which is optically active, the structural identity of the parent ketone is established. Thus 4-methylhexan-2-one (IX) is a minor constituent of *D. (Acanthoclinea) Clarki.*

The structures of derivatives A and D are being investigated.

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SUMMARY

Previous investigations of Dolichoderine ant extractives have resulted in the isolation of a novel group of compounds: the cyclopentanoid monoterpenes. Thus iridomyrmecin has been obtained from the Argentine ant, *Iridomyrmex humilis*, isoiridomyrmecin (iridolactone) from *I. nitidus*, and iridodial from *I. detectus* and *I. conifer*, and from *Tapinoma nigerrimum*. In addition methylheptenone and propyl isobutyl ketone have been identified in association with iridodial. The isolation of dolichodial is now reported from *Dolichoderus (Acanthoclinea) Clarki.* Dolichodial ($C_{10}H_{14}O_2$) readily yields an insoluble red derivative on treatment

with 2,4-dinitrophenylhydrazine. On the basis of degradative and spectroscopic studies, dolichodial is formulated as α-(2-formyl-3-methylcyclopentyl)-acraldehyde. Several compounds obtained in association with dolichodial have been characterized. The relation of dolichodial to the known cyclopentanoid monoterpenes is discussed.

RIASSUNTO

Dolicodial e composti correlati.

Precedenti ricerche su estratti di formiche Dolicoderine si sono risolte nell'isolamento di un gruppo nuovo di composti: i monoterpeni ciclopentanoidi. Così l'iridomirmecina è stata ottenuta dalla Formica argentina *Iridomyrmex humilis*, isoiridomirmecina (iridolattone) da *I. nitidus* ed iridodial da *I. detectus* e *I. conifer* e da *Tapinoma nigerrimum*. Metileptenone e propil-isobutil-chetone sono stati identificati in associazione con iridodial. L'isolamento del dolicodial è ora dato per certo da *Dolichoderus* (*Acanthoclinea*) *Clarki*. Il dolicodial ($C_{10}H_{14}O_2$) produce subito un derivato rosso insolubile in trattamento con 2,4-dinitrofenilidrazina. Sulla base di studi degradativi e spettroscopici il dolicodial è da ritenersi un α-(2-formil-3-metilciclopentil)-acraldeide. Parecchi componenti ottenuti in associazione con dolicodial sono stati caratterizzati. E' discussa la relazione del dolicodial con i monoterpeni ciclopentanoidi noti.

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CHEMICAL AND BIOLOGICAL PROPERTIES OF THE VENOM OF THE IMPORTED FIRE ANT (*SOLENOPSIS SAEVISSIMA* VAR. *RICHTERI* FOREL) AND THE ISOLATION OF THE INSECTICIDAL COMPONENT

The imported fire ant is widely distributed throughout the southeastern United States where it is well known because of its sting. Ants in the field and in the laboratory have been observed to readily attack diverse insect species and rapidly immobilize them by repeated stings. The multiple stings almost invariably result in the death of the insect.

In a preliminary note, Blum *et al.* (1958) reported on some physical and chemical properties of fire ant venom. They showed that the venom of the fire ant had a broad spectrum of biological activity. More recently, Adrouny *et al.* (1959) have isolated from extracts of the fire ant, a component of the venom, which has been characterized as a tertiary amine with strong hemolytic properties. The present paper reports, in detail, various properties of fire ant venom and describes the isolation of the insecticidally active component. This component has been shown to be identical to the hemolytic compound isolated by Adrouny *et al.* (1959).

PHYSICAL AND CHEMICAL PROPERTIES OF FIRE ANT VENOM

Venom collected from the sting of the fire ant is usually water clear and contains a small proportion of suspended droplets. These droplets usually settle from the main phase and constitute from 5 to 7 per cent of the total volume. The venom of a small percentage of the workers examined is extremely heterogeneous and presents a « milky » appearance. In contrast to the almost water-clearness of most venom samples, about 8 per cent of the workers secreted

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venom that is milky in appearance. Twenty to 75 μg. of venom can be obtained from individual ants. However, dissections of the poison glands of ants which had been « milked dry », reveal that their main poison glands still contain a large quantity of venom. This probably indicates that the free duct which connects the convoluted collecting duct to the poison sac (Callahan *et al.* 1959) is closed off.

Infrared spectra of whole venom sometimes indicates the presence of a carbonyl group (5,7 μ) which does not appear to be an open chain simple ketone (Bellamy 1954). However, the majority of venom samples contain only C-H absorption. The venom also contains some unsaturation (6.06 μ). The carbonyl-component sometimes detected in the whole venom is present but not always detectable in the minor phase of the venom. Venom samples transferred to capillary tubes and centrifuged at 22,000 G. separate cleanly into two phases and permit collection of either phase with a microsyringe. Infrared examinations indicate that the clear upper phase contains only intense C-H absorption and a weak unsaturation band.

The visually evident two-phase venom originates in the main poison gland. Microscopic examination of the poison apparatus distad of the accessory gland clearly demonstrates that both the droplets and main phase are synthesized in the main poison gland. This fact is consistent with the observation by Callahan *et al.* (1959) that the main poison gland is composed of two distinct histological areas. Infrared examinations of the contents of the main poison gland dissected free of the accessory gland reveal a spectrum which is identical to that of the whole venom. The carbonyl absorption, which was sometimes observed, originates from the water-clear fluid in the accessory gland. The infrared spectrum of the accessory gland contents in carbon tetrachloride clearly demonstrates the presence of the carbonyl group. The inconsistent carbonyl absorption observed in whole venom samples is probably due to the low percentage of the accessory secretion present in the whole venom.

Components of the venom form complexes with the aniline dye rhodamine B which can be seen readily under the ultra-violet light. Therefore, it is possible to chromatograph the venom and to detect the rhodamine-complexing compounds present. The most successful chromatographic system was a 4:1 mixture of chloroform-methanol on silicic acid impregnated paper. The venom can be separated into three distinct components by employing this system for a six hour ascending run. Two of these components are present in equally small amounts. The third component streaks over a large area and probably represents the clear main phase present in the venom. The rhodamine-complexes of the minor spots fluoresce red under ultra-violet whereas the streaked major component fluoresces blue-violet. The two minor components are present primarily in the suspended droplets in the clear fluid. The contents of poison glands dissected free of the accessory gland also present this three component picture. Therefore, the accessory gland secretion is either in too low a concentration to be detected or does not produce a complex with the chromophoric agent.

BIOLOGICAL PROPERTIES OF FIRE ANT VENOM

Blum *et al.* (1958) have described insecticidal, antibacterial and fungicidal activity of fire ant venom. Adrouny *et al.* (1959) have isolated a hemolytic component from fire ants which appears to be one of the main components of the venom. These properties together with its necrotic activity at the site of an ant sting (Caro *et al.* 1957) demonstrate that the venom has a broad spectrum of biological activity. We have also found that the venom is phytotoxic to certain plants.

Bean leaves which have been dipped in a suspension of venom in distilled water (80 μg/ml) develop a marked chlorosis in 48 hours and present a mosaic-like appearance. Within 72 hours the leaves shriveled and dropped from the plants.

The insecticidal, fungicidal and batectericidal activity of the venom are the properties of the main poison gland fluids; accessory gland extracts are completely inactive. The insecticidal activity of fire ant venom apparently arises from the major component of whole venom. This component which can be collected in microsyringes after centrifugation of the venom is active against all species studied although its speed of action varies from species to species. The sensitivity of the fruit fly, *Drosophila melanogaster* Meig., made this insect an ideal species for bio-assay of fire ant fractions prepared in a program which resulted in the isolation of the insecticidal component of the venom.

ISOLATION OF THE INSECTICIDALLY ACTIVE COMPONENT OF FIRE ANT VENOM

Whereas residues prepared from extracts of whole fire ants are insecticidally active against fruit filies, extracts of fire ants from which the poison glands have been removed are completely inactive. The insecticidal activity observed with fire ant extracts thus derives from the poison glands and insecticidal bio-assays, therefore, indicate the presence or absence of components of the venom.

Approximately 1200 gm of fire ants were macerated in distilled water and then distilled with super-heated steam for 96 hours. The steam distillate was exhaustively extracted with ethyl ether and the ethereal solution was dried over $MgSO_4$. The ether was distilled off under reduced pressure and the remaining viscous oil was dissolved in a small amount of benzene. This solution was strongly insecticidal. The benzene solution was chromatographed on a silicic acid column employing benzene as a percolate and followed by 1, 2, and 5 per cent ethanol in benzene. Maximum insecticidal activity occurred in the 2 and 5 per cent ethanol fractions which were then combined. The solvent was removed under vacuum and the viscous fluid remaining taken up in a small volume of chloroform and chromatographed on silicic acid treated paper. The blue-violet rhodamine complexing area was located and eluted from non-rhodamine treated

papers with acetone. After removal of the acetone a clear odorless fluid remained (50 mg.) which exhibited insecticidal activity. Infrared examination demonstrated tha this compound had the spectrum as the main phase of the venom.

The insecticidally active material is heat stable and soluble in all organic solvents tested. Empirical analysis indicated the presence of carbon, hydrogen and nitrogen, and demonstrated that the compound had a high molecular weight. The compound tested positively for an alkaloid and a hydrochloride was prepared (m. 143-144° C.). The hydrochloride was also insecticidally active. This compound was compared to and found to be identical to the hemolytic factor isolated by Adrouny *et al.* (1959).

The free base is 2 to 3 times as toxic to fruit flies as DDT and its insecticidal properties are now being studied.

The free base has a molecular weight of around 530. The elements carbon, hydrogen and nitrogen make up around 90 % of the molecular weight of the free base. Based on several analyses, this ant derived natural product contains a carbon-hydrogen composition approximating $C_{35}H_{73}$ with one atom of nitrogen.

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SUMMARY

The venom of the imported fire ant (*Solenopsis saevissima* v. *richteri* For.) consists of at least three components which originate in the main poison gland. A fourth component originates in the accessory gland. The main component of the venom consists of a high molecular weight nitrogen-containing compound which is probably an alkaloid. Infrared examinations indicate that this compound is unsaturated. The secretion from the accessory gland contains a carbonyl species which does not appear to be a straight chain compound. The main component of the venom displays the insecticidal properties identified with the venom.

RIASSUNTO

Proprietà chimiche e biologiche del veleno della formica Solenopsis saevissima *var.* richteri *For. e isolamento del componente insetticida.*

Il veleno della formica *Solenopsis saevissima* var. *richteri* For. consiste di almeno tre componenti che hanno origine nella principale glandola del veleno. Un quarto componente ha origine nella glandola accessoria. Il componente principale del veleno è formato da un composto contenente azoto ad alto peso molecolare che è probabilmente un alcaloide. Esami all'infrarosso indicano che questo composto non è saturato. La secrezione della glandola accessoria contiene un carbonile. Il principale componente del veleno rivela le proprietà insetticide identificate nel veleno.

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